COMPLEMENTARY GENES FOR CHLOROPHYLL DEVELOPMENT IN MAIZE AND THEIR LINKAGE RELATIONS[1]

E. W. LINDSTROM
Iowa State College, Ames, Iowa

Received March 22, 1924

TABLE OF CONTENTS

	PAGE
INTRODUCTION	305
Experimental report	306
Chlorophyll relations	306
Linkage relations	311
Linkage of W_1 and Y	311
Linkage of W_2 and R	314
Gene W_3 independent of W_1, W_2, C and L_g	318
DISCUSSION	319
SUMMARY	321
LITERATURE CITED	321
APPENDIX—Tables 9 to 14	322

Whenever a hereditary character develops as the resultant of several genetic factors it becomes of immediate interest to determine the nature of this factorial interaction. Whether this action is "supplementary" or "complementary" is an important consideration. The former bespeaks a different type of chemical (or physiological) behavior, and perhaps evolutionary origin of the chromosomes than does the latter. For example, an F_2 ratio of 15 : 1 or 63 : 1 indicates at least a different chemical relationship between the factors than does a 9 : 7 or 27 : 37 ratio. The chlorophyll characteristics of maize provide a fertile field of research that sheds much light on such problems.

This report is concerned with an intensive study of one phase of the chlorophyll situation in maize, that involving the well known albino seedlings. Technically, albino seedlings are those devoid of the green components of chlorophyll as well as of the yellow xanthophyll or carotinoid pigments. They are distinguished from the virescent-white seedlings, which they simulate in the early stages of growth, by the fact that the virescent-whites ordinarily assume an unmistakable green color soon after emerging. The albino seedlings never become green, although

[1] Paper No. 3, Department of Genetics, IOWA STATE COLLEGE, Ames, Iowa. Presented before the Joint Genetics Sections of the BOTANICAL SOCIETY OF AMERICA and the AMERICAN SOCIETY OF ZOÖLOGISTS meeting at Cincinnati, Ohio, December 27, 1923.

some types may show very faint traces of pale green on the coleoptile. Virescent-whites may grow to full maturity; albinos never do.

Earlier reports (LINDSTROM 1918, 1921) have described the mode of inheritance of the albino seedlings that were due to a single recessive gene. Evidence has been at hand for several years that such white seedlings from different sources were genetically different, although phenotypically similar. Three such types are reported herein. Their factorial interactions and their linkage relations are satisfactorily explained.

EXPERIMENTAL REPORT

Chlorophyll relations

Crosses were made between two inbred stocks of corn, both carrying the recessive albino seedlings. One pedigree culture involved a single factor for white seedlings that had been carried for ten generations of controlled breeding. The other had its origin in some commercial yellow flint corn received from Doctor W. E. CASTLE in 1918, since which time it has been inbred.

Several successful crosses were made in 1920 between green plants heterozygous for the single known albino gene (W_1w_1) and others from the yellow flint cultures which had been giving 9 : 7 ratios of green to albino seedlings. The progenies of three such crosses have been followed through the F_1, F_2 and F_3 generations.[2]

In every case the F_1 plants were normal green (table 1). This at once indicated that the albinos from the two sources were genetically different.

TABLE 1

F_1 data from crossing heterozygous green plants involving two and three pairs of chlorophyll factors.

	PEDIGREE NUMBER	PARENTAL GENOTYPES	F_1 SEEDLINGS
Cross No. 1	4103–20×4265–17	W_1w_1 *yy* R^{g_7r} *AA CC* P_rP_r *ii* L_gl_l × W_2w_2 *YY* $r^{r}r^{g}$ *AA cc* P_rP_r *ii* L_gL_g	118 green
Cross No. 2	4103–21×4265–24	W_1w_1 *yy* R^{g_7r} *AA CC* P_rP_r *ii* L_gl_g × W_2w_2 *YY* r^{g_7g} *AA cc* P_rP_r *ii* L_gL_g	180 green
Cross No. 3	4266–4×4103–14	W_2w_2 W_3w_3 *YY* r^{g_7g} *AA CC* P_r P_r *ii bb* p_1p_1 × W_1w_1 *yy* R^{g_7r} *AA CC* P_rP_r *ii Bb* p_1p_1	106 green

[2] A portion of the F_1 ears were self-pollinated by Prof. J. B. WENTZ, IOWA STATE COLLEGE, in connection with some other problems in genetics. Mr. W. A. CARVER, graduate assistant in genetics, also aided in some of the self-pollination work.

A relatively large number of these fully green F_1 plants were self-fertilized. Being crosses of flint and dent varieties, they were very vigorous. Their seedling progenies were grown in the greenhouse. A large F_2 generation was also grown.

Crosses No. 1 and No. 2 were each found to involve only two different albino genes. The F_2 data as well as the linkage relations prove this fact, conclusively. The parental genetic formulae involved might be given as follows:

Cross No. 1, $W_1w_1\ W_2W_2 \times W_1W_1\ W_2w_2$
Cross No. 2, $W_1w_1\ W_3W_3 \times W_1W_1\ W_3w_3$

Under such circumstances the green F_1 plants would be of four types in equal numbers as follows:

Cross No. 1	*Cross No. 2*
$W_1W_1\ W_2W_2$	$W_1W_1\ W_3W_3$
$W_1w_1\ W_2W_2$	$W_1w_1\ W_3W_3$
$W_1W_1\ W_2w_2$	$W_1W_1\ W_3w_3$
$W_1w_1\ W_2w_2$	$W_1w_1\ W_3w_3$

When such F_1 plants are selfed, three distinct F_2 seedling progenies should be apparent. One-fourth of the progenies in either cross would consist entirely of green seedlings; one-half of 3 : 1 ratios of green to white seedlings; and the remaining one-fourth should segregate into 9 greens : 7 whites if the factorial interaction of the two genes was complementary in nature, and if they were also inherited independently of each other. Such proved to be the case.

A total of 119 F_2 seedling progenies was tested on a relatively large scale. The complete data are listed in the appendix (tables 9 and 10). A summary of the progenies has been arranged in table 2.

TABLE 2

Summary of F_2 seedling progenies involving two complementary genes. Details in tables 9 and 10.

	PROGENIES OF ALL GREEN	PROGENIES OF 3 GREEN : 1 ALBINO	PROGENIES OF 9 GREEN : 7 ALBINO	PROBABILITY P
Cross No. 1	12	27	14	
Cross No. 2	23	30	13	
Total	35	57	27	0.56
Expected	*30*	*59*	*30*	

This summary exhibits a close approximation to the expected 1 : 2 : 1 ratio of the three types of seedling progenies. The deviations can be

reasonably due to errors of random sampling since P has a value of 0.56. Accordingly it becomes evident that the two genes for albino seedlings involved in each of crosses No. 1 and No. 2 are genetically different. It is also apparent that the factorial interaction of their dominant allelomorphs is complementary.

A glance at the detailed data in tables 9 and 10 gives further verification for the hypothesis, since all the individual ratios are surprisingly close to expectation. A total of all the 9 : 7 ratios in these tables gives 2036 greens : 1542 albinos, where 2013 : 1565 are expected. This exhibits a deviation of 23, which is only 1.2 times its probable error. From such data one can be reasonably certain that W_1 and W_2, or W_1 and W_3 are independently inherited. However, their linkage relations, discussed later, serve as a real verification of this fact.

The most interesting developments arose in cross No. 3, which was a cross between green plants segregating for 3 : 1 and 9 : 7 ratios. The parental genotypes in this case may be written as follows:

$$W_1W_1\ W_2w_2\ W_3w_3 \times W_1w_1\ W_2W_2\ W_3W_3$$

Such a situation calls for an F_1 generation of green plants comprising eight different genotypes in equal proportions as follows:

$W_1W_1\ W_2W_2\ W_3W_3$	All green
$W_1w_1\ W_2W_2\ W_3W_3$	3 green : 1 white
$W_1W_1\ W_2w_2\ W_3W_3$	3 green : 1 white
$W_1W_1\ W_2W_2\ W_3w_3$	3 green : 1 white
$W_1w_1\ W_2w_2\ W_3W_3$	9 green : 7 white
$W_1w_1\ W_2W_2\ W_3w_3$	9 green : 7 white
$W_1W_1\ W_2w_2\ W_3w_3$	9 green : 7 white
$W_1w_1\ W_2w_2\ W_3w_3$	27 green : 37 white

If the third gene is acting in a complementary fashion like the others, a triheterozygous green F_1 plant should produce an F_2 seedling progeny of 27 green : 37 white or albino seedlings. This is a new sort of chlorophyll ratio, one in which there is a preponderance of the recessive, white seedlings. Eleven such progenies were found among the 72 F_2 progenies that were tested (see table 3).

The eleven progenies gave a total of 1094 green and 1422 albino seedlings. The theoretical expectancy, based on a 27 : 37 ratio, typical of three pairs of independent, complementary genes, is 1061 : 1455, a deviation of 33, which is only twice as large as the probable error. In this case we have the usual slight excess of the green class, a phenomenon which is characteristic of large counts involving recessive defects in maize. A slightly lower germination or viability of the recessive class, coupled

TABLE 3

The 72 F_2 seedling progenies of cross 3 arranged in four classes according to chlorophyll behavior.

F_3 EAR NUMBER	ALL GREEN	F_3 EAR NUMBER	3 GREEN : 1 ALBINO		F_3 EAR NUMBER	9 GREEN : 7 ALBINO		F_3 EAR NUMBER	27 GREEN : 37 ALBINO	
201	86	205	168	53	202	121	75	209	90	135
217	138	207	159	52	203	104	67	213	74	123
225	147	210	261	81	204	116	91	215	67	80
227	126	211	127	28	206	136	126	216	66	79
234	148	218	100	43	208	159	113	219	127	184
249	66	223	120	35	212	148	103	230	133	163
257	133	224	201	61	214	123	76	237	179	191
264	150	226	126	32	220	99	63	248	42	44
		228	186	62	221	129	83	261	112	156
		229	116	32	222*	162	174	268	87	110
		232	267	89	231	174	113	273	117	157
		239	265	83	233	114	93			
		240	171	65	235	173	134			
		241	174	60	236	216	165			
		253	297	119	242	179	132			
		254	156	38	243	123	118			
		258	101	33	244	185	139			
		259	137	42	245	148	111			
		260	131	35	246	75	67			
		262	164	55	247	96	77			
		266	111	41	250	93	64			
		267	88	29	251	140	110			
		269	114	38	252	84	74			
		271	102	34	255	79	53			
		272	95	26	256	91	44			
					263	179	134			
					265	229	166			
					270	102	64			
Total	994		3937	1266		3777	2829		1094	1422
Expected	*994*		*3902*	*1301*		*3716*	*2890*		*1061*	*1455*
Dev.	0		1.7			2.2			2.0	
P.E.										

* Determined by linkage tests to be a 9 : 7 ratio.

with the inevitable small percentage of contamination by stray pollen (which invariably adds to the normal class) could easily account for the slight excess of the dominant class, were this necessary. Obviously the deviation of 33 individuals in this case falls within reasonable limits of error due to random sampling. However, the progeny test in cross No. 3 gives a much safer and surer criterion of the factorial relations (table 4).

Before leaving table 3, it is worth noting that the individual 3 : 1 and 9 : 7 ratios therein are based on sufficiently large numbers so as to cause no difficulty in differentiating them from one another or from the 27 : 37 progenies. It was not thought necessary to calculate the individual probable errors, since mere inspection affords a reasonable certainty of their correct classification. Accordingly only the totals for each type of ratio were tested for their agreement with the expected results. In no case was the deviation beyond reasonable limits of divergence.

A summary of the F_2 seedling progenies of cross No. 3 is afforded in table 4.

TABLE 4

Summary of F_2 seedling progenies involving three complementary genes. Details in table 3.

	PROGENIES OF ALL GREEN	PROGENIES OF 3 GREEN : 1 ALBINO	PROGENIES OF 9 GREEN : 7 ALBINO	PROGENIES OF 27 GREEN : 37 ALBINO	PROBABILITY
Cross No. 3..	8	25	28	11	
Expected.....	*9*	*27*	*27*	*9*	
Deviation...	−1	−2	+1	+2	$\chi^2=0.740$

If the factorial hypothesis is correct there should be among these 72 progenies one-eighth of the total showing all green, three-eighths giving 3 : 1 ratios, three-eighths giving 9 : 7 ratios and one-eighth showing 27 : 37 ratios. The actual data show very little deviation from this expectancy. The closeness of fit gives a value for χ^2 of 0.740 which means a value for P of over 0.80. Such an agreement most certainly substantiates the theory that in this material the inheritance of the green pigment, chlorophyll, as evidenced by the action of the recessive, albino character, can be explained on the basis of three complementary genes.

Accordingly, normal green pigment can be said to be the product of the dominant genes, $W_1W_1W_2W_2W_3W_3$ acting in a complementary fashion. The homozygous recessive condition of any one gene, or two, or three together, is sufficient to inhibit chlorophyll development. It might be noted at this time that the action of each of the three recessive genes in this case is slightly different, although these differences are so minute that they are easily overlooked. Gene w_2w_2 produces the purest type of white seedling. The color is a paper white. With optimum conditions of growth genes w_3w_3 and w_1w_1 apparently permit a very slight development of chlorophyll, especially on the coleoptile. In no case, however, does real

green pigment develop in the leaves, so that these so-called albinos might be phenotypically confused with extreme types of virescent-white seedlings. Incidentally it might be added that these three types of albinos have been crossed with pedigreed virescent-white material and the resulting seedlings were entirely green.

Linkage relations

That the three complementary genes W_1, W_2 and W_3 are inherited independently of each other is practically proven by the close approximation to the theoretical 27 : 37 ratio of green to albino plants produced by a triheterozygous green plant. In addition a large amount of data has been accumulated to prove that W_1, W_2 and W_3 belong in separate linkage groups. Fortunately these linkage groups are involved in cross No. 3 whose parental genotypes were as follows: W_1W_1 W_2w_2 W_3w_3 YY r^gr_g AA CC bb $p_1p_1 \times W_1w_1$ $W_2W_2W_3W_3$ yy R^gr^r AA CC Bb p_1p_1.

Linkage of W_1 and Y

The yellow endosperm factor Y in this cross is one that comes from a pure, inbred yellow flint variety. Fortunately, this type of yellow endosperm when crossed with white endosperm gives a single-factor relationship permitting a reasonably clean-cut F_2 distribution of yellow and white grains. Even here, however, some slight difficulty is occasionally experienced in separating a small percentage of the kernels. Most of the F_2 ears nevertheless show an unmistakable 3 : 1 ratio of yellow to white grains. These afford a critical test of the linkage between W_1 and Y.

This linkage may be the same as that mentioned by KEMPTON (1917). It is identical with that reported by STROMAN (1924), although the data presented herewith do not give exactly the same intensity of linkage as was found by STROMAN.

Of the 72 F_2 ears in cross No. 3, there were 37 that possessed aleurone color (3 purple : 1 colorless), and 35 that were entirely colorless as regards aleurone. The latter afford a good means of testing the endosperm-chlorophyll linkage, since all of them had approximately 75 percent yellow and 25 percent white grains.

Among the 35 ears devoid of aleurone color there were 14 that segregated for 3 green : 1 albino; 14 for 9 green : 7 albino; and 4 for 27 green : 37 albino. The remaining 3 ears gave only green seedlings. Linkage between W_1 and Y was observed in 8 of the 3 : 1 ears, 7 of the 9 : 7 ears, and in all of the 4 ears giving the 27 : 37 chlorophyll ratio. The individual counts of these 16 ears are arranged in table 11. The data on the 13 ears

that did not show any linkage between Y and albino genes are recorded in table 12.

A summary of the linkage progenies that appear in table 11 is arranged in table 5, as follows:

TABLE 5

Summary of F_2 data showing linkage between W_1 and Y, giving 35 percent crossing over. Cross No. 3. Details in table 11.

GENOTYPE OF F_1 MOTHER PLANT	PARENTAL TYPES *YW* AND *yw*		NON-PARENTAL TYPES *Yw* AND *yW*		Dev. / P. E
	Observed	Expected	Observed	Expected	
$YW_1 \cdot yw_1$	1211	1214	496	493	0.2
$YW_1w_2 \cdot yw_1W_2$ or $YW_1w_3 \cdot yw_1w_3$ . . .	789	796	546	539	0.6
$YW_1w_2w_3 \cdot yw_1W_2W_3$	435	420	390	405	1.5

In this table, as in others to follow, the method of grouping the parental against the non-parental classes has been followed. There is an obvious advantage in such a grouping whenever a defective character is concerned, one that may cause a lower viability or germination of the individuals possessing it. Under such circumstances it is not only legitimate but essential to group a normal and a defective character into one class. In this particular case both the parental and non-parental classes comprise a green and an albino type.

Thus, in calculating the deviations from a dihybrid distribution the 9- and 1-classes of the F_2 phenotypes are grouped against the two 3-classes, giving, with independent inheritance, a 10 : 6 ratio. With various degrees of linkage this 10 : 6 ratio is modified. The probable error is that calculated on such a modified basis.

The same procedure is followed for a trihybrid and for a tetrahybrid. With complementary action of two chlorophyll genes plus an endosperm gene, the trihybrid situation becomes a modified form of the 27 : 9 : 21 : 7 ratio. When the parental and non-parental classes of phenotypes are grouped this ratio becomes a modified 34 : 30, the modification being dependent on the degree of linkage. The tetrahybrid situation (in this case due to $Yy\ W_1w_1\ W_2w_2\ W_3w_3$) becomes an 81 : 27 : 111 : 37 phenotypic ratio which, when grouped according to parental and non-parental types, becomes a modified 118 : 138 ratio.

These relationships may be stated for this problem in general terms, involving the gametic ratio ($r : s$), as follows:

	Parental types	*Non-parental types*
Dihybrid	$4r^2+2(2rs+s^2)$	$2(2rs+s^2)$
Trihybrid	$13r^2+7(2rs+s^2)$	$3r^2+9(2rs+s^2)$
Tetrahybrid	$43r^2+25(2rs+s^2)$	$21r^2+39(2rs+s^2)$

Accordingly, with any degree of linkage (percentage of crossing over) it merely becomes necessary to determine the gametic ratio, in other words, the values of r and s. In this way the theoretical or expected frequencies may be found and the deviations therefrom calculated.

In regard to the probable errors of such a method of expressing linkage intensities it seems legitimate to use an extension of the ordinary method of calculating probable errors, as is done for 9 : 7 and 27 : 37 ratios that involve complementary interaction of factors. Such probable errors are based on the general formula, $.6745\sqrt{p\times q\times n}$, in which p and q are the elements of the ratio whose sum totals 1.000. With this particular formula for the probable error the actual deviations are calculated from the expected frequency expressed in terms of absolute numbers.

Probable-error formulae under such circumstances may be written as follows:

$$\text{P. E.}_{\cdot 10:6} = .6745\sqrt{[4r^2+2(2rs+s^2)]\times[2\ (2rs+s^2)]\times n}$$

$$\text{P. E.}_{\cdot 34:30} = .6745\sqrt{[13r^2+7(2rs+s^2)]\times[3r^2+9(2rs+s^2)]\times n}$$

$$\text{P. E.}_{\cdot 118:138} = .6745\sqrt{[43r^2+25(2rs+s^2)]\times[21r^2+39(2rs+s^2)]\times n}$$

In case of the W_1Y linkage, for example, the data fit very well the hypothesis of 35 percent crossing over between YW_1 and yw_1 (see table 5). With this amount of crossing over, the gametic ratio ($r : s$) is 1.8571 : 1. In this cross we are dealing with the 'coupling' phase of the linkage, since Y and W_1 came from one parent.

The probable errors for the three sorts of distributions in table 5 with 35 percent crossing over have been calculated as follows:

$$\text{P. E. of dihybrid} = .6745\ \sqrt{.711\times .289\times n} = \sqrt{.0935n}$$

$$\text{P. E. of trihybrid} = .6745\ \sqrt{.596\times .404\times n} = \sqrt{.1095n}$$

$$\text{P. E. of tetrahybrid} = .6745\ \sqrt{.509\times .491\times n} = \sqrt{.1137n}$$

An inspection of the data in table 5 shows clearly that the three distributions therein agree remarkably well with the expected results based on 35 percent crossing over between W_1 and Y. The largest deviation is

only 1.5 times as large as its probable error. If the data are compared with the expected results with independent inheritance, the deviations show beyond doubt that independent inheritance of W_1 and Y is impossible. The deviation in the case of the dihybrid involving these two factors proves, for example, to be eleven times larger than its probable error.

A glance at the individual progenies from which the summary in table 5 has been arranged, leaves no uncertainty as to their similarity (see table 11). Accordingly, there should be no hesitation in stating that W_1 and Y belong in the same linkage group, a group which already has three other factors, making a total of five factors, Y-P_l-S_m-F_i-W_1.[3] Incidentally, STROMAN (1924) has reported that W_1 and P_l show linkage, but the data are very limited.

In order to present a complete record of this experiment it is necessary to give the data for those progenies showing no linkage between Y and the albino genes (in this case w_2 and w_3). Such a record is presented in table 12. Here there are 13 progenies segregating for albino seedlings, 6 on a 3 : 1 basis and 7 on a 9 : 7 basis. Obviously there would be no 27 : 37 ratios in this table. The data in table 12 agree very well with a theoretical distribution based on independent inheritance between Y and either W_2 or W_3.

Linkage of W_2 and R

This is a new linkage whose details have not as yet been reported. Cross No. 3 carried the R factor for aleurone development as follows:

F_1 genotype $RW_2w_1W_3 \,.\, rw_2W_1w_3$ $(AA\ CC\ P_rP_r\ ii)$

In this cross there were 37 selfed F_1 ears that came from purple F_1 grains of the crossed ear. In other words there were 37 F_2 distributions of 3 purple : 1 colorless. Of these, 5 did not segregate for albino seedlings; 11 gave 3 : 1 ratios; 14 gave 9 : 7 ratios; and 7 gave 27 : 37 ratios of green to albino seedlings. Accordingly there will be dihybrid, trihybrid and tetrahybrid F_2 generations involving aleurone color (Rr) and the various chlorophyll factors.

The detailed progenies of these three sorts are listed in two tables. In table 13 are those progenies showing a linkage between the aleurone

[3] Since this paper was written an article by DEMEREC (1923) has appeared, adding two other albino genes, W_5 and W_6. Each of these shows about 25 percent crossing over with Y, and apparently are located on either side of this endosperm factor. Thus, this group has in reality seven different genes, since it is not likely that W_1 is identical with either W_5 or W_6, their linkages with Y rendering this impossible in all likelihood.

and chlorophyll factors. In table 14 are the progenies that showed no linkage, obviously because W_2 was not concerned.

In table 13 there are 3 progenies segregating for 3 greens : 1 albino that show an unmistakable linkage. In addition there are 10 progenies with 9 greens : 7 albinos, and 7 that give 27 : 37 ratios on chlorophyll. A summary of these 20 progenies has been arranged in table 6.

TABLE 6

Summary of F_2 data showing linkage between W_2 and R giving 15.4 percent crossing over. Cross No. 3. Details in table 13.

GENOTYPE OF F_1 MOTHER PLANT	PARENTAL TYPES *RW* AND *rw*		NON-PARENTAL TYPES *Rw* AND *rW*		Dev. / P. E
	Observed	Expected	Observed	Expected	
$RW_2 \cdot rw_2$	805	824	155	136	2.6
$RW_2W_1 \cdot rw_2W_1$ or $RW_2W_3 \cdot rw_2w_3$ . . .	1769	1735	689	723	2.2
$RW_2w_1W_3 \cdot rw_2W_1w_3$	1015	1001	676	690	1.0

In table 6 the same procedure has been followed, namely, of grouping the parental against the non-parental combinations. From this grouping the deviations and probable errors have been calculated exactly as was done in the case of the W_1-Y data.

In this R-W_2 situation there is somewhat more variability in the progenies. Most of this is probably due to the fact that not only is W_2 linked with R, but also with a defective type of endosperm. This linkage between W_2 and D_f has been reported in a previous paper (LINDSTROM 1923). A situation in which two lethal factors (one for endosperm and the other for chlorophyll) are competely linked is certain to disturb the viability of that class more than of the other. Notwithstanding this difficulty, the data in table 13 and table 6 fit a hypothesis of 15.4 percent crossing over between W_2 and R reasonably well.

In determining the goodness of fit of these data, the probable errors were calculated with a gametic ratio of 5.5 : 1, which gives 15.4 percent breaks in the linkage. These probable errors, which are based on modifications of the 10 : 6, 34 : 30 and 118 : 138 ratios, were determined to be:

P. E. of dihybrid $= .6745\sqrt{.858\times.142\times n} = \sqrt{.0554n}$

P. E. of trihybrid $= .6745\sqrt{.706\times.294\times n} = \sqrt{.0944n}$

P. E. of tetrahybrid $= .6745\sqrt{.592\times.408\times n} = \sqrt{.1099n}$

Using this method on the data in table 6, it will be seen that, while the deviations are not beyond the limits of a reasonable fit, they are somewhat larger than they should be ideally. As a matter of fact the poorest fit occurs in the 3 : 1 progenies ($RW_2 . rw_2$) and in this case the divergence from the expected can be attributed to one of the three progenies comprising this total. The F_1 ear No. 210 is the major cause of the poor fit. Whether its deviations are due to pollen contamination (the aleurone ratio is somewhat abnormal) or to errors of random sampling it is impossible to determine. Inasmuch as all the other progenies are within reasonable limits of the hypothesis we may perhaps believe that a deviation 2.6 times as large as its probable error should be expected occasionally.

In order to give the complete data on the relation between aleurone and chlorophyll factors in cross No. 3, the 12 progenies showing no linkage with *R* have been tabulated in table 14. In this case the progenies involve the factors W_1 and W_3 which are not linked with *R*. There is no semblance of linkage among these 12 progenies.

Additional data on the W_2-*R* linkage were afforded in cross No. 1 which carried the following factors: $w_1W_2RAC.W_1w_2rAc$. Some of the F_2 ears of this cross possessed aleurone ratios of 9 purple : 7 colorless, and in addition some segregated for 3 : 1 and others for 9 : 7 chlorophyll ratios. Thirteen such progenies have been listed in table 7.

There are in this table, 8 progenies from triheterozygous F_1 plants of the composition $W_2RC.w_2rc$, and 5 progenies from F_1 plants of the genotype $w_1W_2RC.W_1w_2rc$. With linkage between W_2 and *R* on a basis of 15.4 percent crossing over, some rather peculiar ratios are observed. These, however, agree very well with the linkage hypothesis, the deviations being within reasonable limits. For example, a total of the 3 : 1 progenies (on chlorophyll) gives 675 of the parental classes and 276 of the non-parental, where 671 and 280, respectively, are expected. This is a deviation of only 4. The 9 : 7 progenies (on chlorophyll) total 399 of the parental classes and 228 of the non-parental, where 401 and 226 are expected, a deviation of only 2. These small deviations obviously indicate excellent agreement with the hypothesis.

Accordingly, from a total of 6687 individuals grown from two separate crosses (Nos. 1 and 3) involving the linkage between W_2 and *R*, it can be stated with reasonable certainty that these two factors are fairly closely linked. The data in both crosses give a crossover percentage of 15.4 with which there is good agreement.

It is worth noting that the aleurone factor involved in this linkage has been identified as *R* mainly because of the seedling stem-color determina-

TABLE 7

Individual F_2 seedling progenies showing linkage between R aleurone and W_2 chlorophyll factors when aleurone ratio is 9 : 7 (AA Cc Rr). Cross No. 1.

F_3 EAR NUMBER	FROM PURPLE SEEDS		FROM COLORLESS SEEDS		CHLOROPHYLL RATIO	DEV. / P. E.
	Green	Albino	Green	Albino		
2	84	3	35	34	3 : 1	
6	69	3	26	14	3 : 1	
14	52	2	27	14	3 : 1	
16	59	3	30	18	3 : 1	
21	49	5	33	14	3 : 1	
26	65	3	34	29	3 : 1	
27	72	11	32	27	3 : 1	
28	54	5	24	21	3 : 1	
Total	504	35	241	171		0.4*
Expected	*484*	*51*	*229*	*187*		
8	51	21	24	24	9 : 7	
9	51	32	25	41	9 : 7	
13	43	24	21	25	9 : 7	
23	54	19	24	33	9 : 7	
24	50	13	25	27	9 : 7	
Total	249	109	119	150		0.3†
Expected	*240*	*113*	*113*	*161*		

* Calculated on 27 : 9 : 21 : 7 or 34 : 30 basis, modified by linkage.
† Calculated on modified 81 : 63 : 63 : 49 ratio, or 130 : 126 basis.

tions that are characteristic of the multiple allelomorphic situation R^r, R^g, r^r, r^g (EMERSON 1921). All of the data show linkage with seedling stem-color (red and non-red) whenever w_2 is present. In cross No. 3 there was little or no mottling of aleurone when R was carried over from the male parent, probably because of the S factor (KEMPTON 1919).

Incidentally it might be stated that a large amount of data is already at hand verifying this linkage from another angle. In this case W_2 has been found to be linked with the *luteus* factor l (involved in the development of the xanthophyll and carotinoid pigments of maize). The details of this linkage are reserved for a future report.

Thus, the linkage group *R-L-G* has the proven addition, W_2. To these four factors must be added another. This is the recessive endosperm defect (LINDSTROM 1923) that is completely (or almost so) linked with W_2. The genetic symbol for this defective endosperm character may be given as d_f, referring not only to its defective nature but also to the fact that it originated in flint corn.

Two more factors have been suggested as belonging to this group, namely *S* (spotted aleurone) and L_i (lineate leaves). The complete group, accordingly, has a total of seven genetic factors, *R-L-*W_2*-G-*D_f*-S-*L_i. Gene W_2 has been located with respect to *R* and *L* but not to *G*.

Gene W_3 independent of W_1, W_2, *C* and L_g

This third complementary factor for chlorophyll development has not as yet been placed in any of the seven (possibly eight) known linkage groups of maize. Apparently it does not belong in the *Y-*P_l*-*W_1*-*S_m group nor in the *R-L-*W_2*-G-*D_f*-S-*L_i group, as shown by the preceding experiments.

The following data (table 8) taken from cross No. 2 indicate that it is not related to the L_g*-B-*T_s group. In this case W_3 has been tested with the liguleless factor l_g. In neither the 9 : 3 : 3 : 1 nor the 27 : 9 : 21 : 7

TABLE 8

Showing the independent inheritance of W_3 *and* L_g *in* F_2 *progenies in cross No. 2 From* F_1 *genotypes* $W_3l_g \cdot w_3L_g$ *and* $W_3w_3l_g \cdot w_3W_1L_g$.

PEDIGREE NUMBER	CHLOROPHYLL RATIO	GREEN		ALBINO		DEV. / P. E.
		L_g	l_g	L_g	l_g	
123	3 : 1	97	27	26	7	
151	3 : 1	62	22	19	9	
152	3 : 1	76	32	19	11	
158	3 : 1	85	35	20	10	
165	3 : 1	71	26	34	10	
Total		391	142	118	47	.02*
Expected		*393*	*131*	*131*	*44*	
111	9 : 7	85	32	73	12	
131	9 : 7	60	25	45	10	
134	9 : 7	31	11	24	6	
140	9 : 7	128	47	84	31	
141	9 : 7	91	33	101	30	
146	9 : 7	29	15	30	13	
161	9 : 7	67	16	56	19	
Total		491	179	413	121	2.4†
Expected		*508*	*169*	*395*	*132*	

* Calculated on 10 : 6 basis.

† Calculated on 34 : 30 basis.

distributions of table 8 is there any real indication that a linkage exists between W_3 and L_g. Both distributions show a reasonably good fit with

those expected when independent inheritance between these two factor pairs is assumed.[4]

Evidence for the independent inheritance of W_3 and C is also afforded in cross No. 2. Six F_1 ears of this cross possessed the genotype W_3w_1ACR. w_3W_1Acr. With this tetrahybrid situation there should be in F_2 a ratio of 81 green seedlings from purple seeds : 63 whites from purple seeds : 63 greens from colorless seed : 49 whites from colorless seed. The 6 F_2 distributions (Nos. 111, 115, 119, 122, 131, and 134) gave a total ratio of 278 : 178 : 208 : 143 of the classes noted above. This results in 421 individuals with the parental combinations (W_3C and w_3c) and in 386 individuals with the recombination or non-parental classes (W_3c and w_3C). With independent inheritance between W_3 and C, the expected frequencies are 410 and 397, respectively. The deviation of 11 proves to be only 0.9 as great as the probable error. Accordingly, genes W_3 and C are not linked.

DISCUSSION

In the discovery of a 27 : 37 ratio with seedling chlorophyll characters in maize, a second case of complementary factorial interaction becomes known. EMERSON (1918) reported the first occurrence of such a ratio with aleurone color. In this situation genes A, C and R interact to give 27 colored : 37 colorless aleurone grains. While it may be premature to speculate on the meaning of various factorial interactions in any species, it is, nevertheless, interesting to note that in maize both complementary and supplementary action of genetic factors are known. The latter type of factorial behavior occurs with the duplicate genes for zigzag stalks and very likely with duplicate genes for yellow endosperm, giving the characteristic 15 : 1 ratio.

Some one has suggested that the 15 : 1 and 63 : 1 ratios, common in the small grains like wheat and oats, are perhaps indicative of an evolutionary origin by chromosome duplication. May it be that 9 : 7 and 27 : 37 ratios, when they become numerous in any species, indicate a different sort of evolutionary origin, at least for the chromosomes thus involved? Not until the genetic analysis of a species becomes better known, however, can we attempt any such generalizations. At the present time it seems more likely that complementary action of genetic factors is merely characteristic of a certain type of chemical activity within the hereditary complex of the species.

[4] Gene W_3 in this material may be identical with the W_4 factor reported by DEMEREC (1923). Until the exact linkage relations are determined this must rest as a pure assumption.

It is gradually being demonstrated that genetic factors for chlorophyll development in maize are to be found in most, if not all, of the known linkage groups. In this report, such a simple phase of the general chlorophyll situation as that of seedling albinism, involved three independent genes. To these must be added at least five other genes for albinism as reported recently by DEMEREC (1923). Accordingly, eight different genetic factors for albinism in maize are known, and before long several more will be added to this list. At least five of these known albino genes are in different chromosomes.

When we consider that a host of other similar factors, as those for virescent-white (several different factors), pale green, yellow, lineate, golden, green-striped, yellow-striped, fine-streaked, fine-striped, *japonica*, white-sheath, zebra and other recessive chlorophyll defects also seem to be located in various chromosomes, it soon becomes evident that all of the maize chromosomes are presumably carrying genes whose most striking expression occurs in the pigment chlorophyll. Accordingly, all of their allelomorphs apparently function together in the genetic development of this pigment. Thus, there are known at present not less than 24 different pairs of factors for chlorophyll development in maize, and the end is not yet in sight.

The same general situation is found in characters like anthocyanin pigment (including aleurone and plant colors), in the various growth characteristics (especially those involving dwarfishness of the plant), and also in the case of endosperm development as is evidenced by the multitude of genes responsible for hereditary defects of the endosperm.

Is it possible that each maize chromosome carries a general determiner for each of the characteristics of the plant noted above, and that these numerous, recessive, mutant genes have merely modified these main centers of activity in the chromosomes? This is not likely when we note that occasionally two recessive chlorophyll defects, for example, show linkage, indicating that they are borne on the same chromosome and probably some distance apart.

It is of course conceivable that the genes which we consider as being responsible for chlorophyll, aleurone or endosperm characters are not all directly nor solely related to these characters, but are rather general chemical determiners whose effect is more or less general. It is difficult to conceive of such a possibility, however, since among the multitude of recessive genes that we already know there are few, if any, that have a second, known, genetic effect. True, there is the occasional complete linkage, so-called, of recessive defects, such as that involving w_2 and d_f, the endosperm defect. But obviously more of such cases would be apparent

if mutant genes had a very general effect on the genetic development of maize. For this reason we are inclined to believe that the numerous chlorophyll, aleurone, and endosperm genes are perhaps specific in nature, although they may have a general physiological effect on the organism. It is also evident that these genes appear to be scattered throughout all of the chromosomes in maize, sometimes several that affect the same character occurring in one chromosome. Eventually, however, we may be driven to the conclusion that each normal chromosome holds centers of genetic activity for all the hereditary characters involved in Mendelian inheritance.

SUMMARY

1. Three different genes for complete albinism in maize seedlings were found to be genetically distinct. Their normal allelomorphs act in a complementary manner to cause the development of normal chlorophyll.

2. Seedling ratios of 3 : 1, 9 : 7 and 27 : 37 green to albino plants occur when mono-, di- or triheterozygous green plants, respectively, are self-fertilized.

3. Experimental data on a large scale with the 27 : 37 ratios derived from green plants of the composition $W_1w_1\ W_2w_2\ W_3w_3$ show such a close approximation to the theoretical expectancy that these three factor pairs must be independently inherited.

4. Verification for this independent inheritance is afforded by certain linkage relations. Linkage tests prove that W_1 belongs in the Y-P_l-S_m group since Y and W_1 show 35 percent crossing over in 3 : 1, and also in 9 : 7 and 27 : 37 combinations with the other albino genes.

5. Gene W_2 falls in the R-L-G linkage group, since a large series of tests prove that R and W_2 are fairly closely linked, giving 15.4 percent crossing over. This linkage was observed in 3 : 1, 9 : 7 and 27 : 37 chlorophyll ratios.

It is also shown that w_2 is the albino gene that is linked with d_f, a factor for defective endosperm. Apparently this is a case of complete linkage.

6. Gene W_3 is independent of the two linkage groups mentioned above. Data are also presented to show that it belongs neither in the B-L_g linkage group nor in the C-I-S_h-W_z group.

7. Probable-error methods for use with F_2 distributions involving linkage are described.

LITERATURE CITED

DEMEREC, M., 1923 Inheritance of white seedlings in maize. Genetics 8: 561–593.

EMERSON, R. A., 1918 A fifth pair of factors, *Aa*, for aleurone color in maize, and its relation to the *Cc* and *Rr* pairs. Cornell Univ. Agric. Exp. Sta. Mem. 16: 225–289.

1921 The genetic relations of plant colors in maize. Cornell Univ. Agric Exp. Sta. Mem. 39: 1–156.

KEMPTON, J. H., 1917 A correlation between endosperm color and albinism in maize. Jour. Washington Acad. Sci. 7: 146–149.

1919 Inheritance of spotted aleurone color in hybrids of Chinese maize. Genetics 4: 261–274.

LINDSTROM, E. W., 1918 Chlorophyll inheritance in maize. Cornell Univ. Agric. Exp. Sta. Mem. 13: 1–68.

1921 Concerning the inheritance of green and yellow pigments in maize seedlings. Genetics 6: 91–110.

1923 Heritable characters of maize. XIII. Endosperm defects—sweet defective and flint defective. Jour. Heredity 14: 127–135.

STROMAN, G. N., 1924 Genetic relations of chlorophyll and anthocyanin seedling characters in maize. Genetics 9: 91–123.

APPENDIX—Tables 9 to 14

TABLE 9

The 53 F_2 seedling progenies of cross No. 1—$W_1w_1\ W_2W_2 \times W_1W_1\ W_2w_2$.

F_1 EAR NUMBER	ALL GREEN	F_1 EAR NUMBER	3 GREEN : 1 ALBINO		F_1 EAR NUMBER	9 GREEN : 7 ALBINO	
4	99	1	114	32	8	75	45
7	44	2	119	37	9	76	73
12	39	3	120	44	13	64	49
15	36	5	87	21	23	78	52
19	132	6	95	17	24	75	40
20	113	10	77	33	32	50	34
36	36	11	118	32	33	64	44
38	29	14	79	16	34	84	54
43	38	16	89	21	35	50	45
45	135	17	64	28	39	59	39
46	117	21	82	19	41	49	46
47	121	22	75	32	44	61	43
		25	95	36	49	71	56
		26	99	32	51	73	60
		27	107	38			
		28	76	26			
		30	84	20			
		31	208	72			
		37	102	38			
		40	63	13			
		42	111	33			
		48	109	40			
		50	107	32			
		52	96	32			
		53	80	31			
		54	93	30			
		55	88	32			
Total	939		2637	837		929	680
Expected	*939*		*2605*	*869*		*905*	*704*
Dev.	0		1.9			1.8	
P. E.							

TABLE 10

The 66 F_2 seedling progenies of cross No. 2—$W_1w_1\ W_3W_3 \times W_1W_1\ W_3w_3$.

F_1 EAR NUMBER	ALL GREEN	F_1 EAR NUMBER	3 GREEN : 1 ALBINO		F_1 EAR NUMBER	9 GREEN : 7 ALBINO	
101	64	102	48	17	111	109	79
104	93	103	106	34	115	69	39
108	78	105	175	63	119	105	64
114	150	106	45	9	122	76	54
116	104	107	111	38	131	85	55
117	129	109	135	56	134	42	30
118	117	110	128	44	140	165	117
120	164	112	94	31	141	124	131
121	146	113	135	42	146	44	43
125	142	123	124	33	157	79	71
129	114	124	123	45	161	82	75
130	130	126	102	28	164	57	47
132	90	127	105	38	166	70	57
133	112	128	81	31			
135	58	138	213	65			
136	75	142	164	48			
137	81	144	96	28			
139	74	145	186	59			
143	65	148	87	26			
147	134	149	105	40			
150	110	151	85	28			
153	131	152	107	30			
163	151	154	109	37			
		155	118	41			
		156	112	39			
		158	121	31			
		159	112	40			
		160	91	33			
		162	91	33			
		165	97	44			
Total	2512		3406	1131		1107	862
Expected	*2512*		*3403*	*1134*		*1108*	*861*
Dev. / P. E.	0		0.2			0.1	

TABLE 11

Individual F_2 seedling distributions with respect to endosperm and chlorophyll factors, showing linkage between factors Y and W_1. Cross No. 3.

F_2 EAR NUMBER	CHLOROPHYLL RATIO	FROM YELLOW SEEDS		FROM WHITE SEEDS	
		Green	Albino	Green	Albino
239	3 : 1	208	54	57	29
241	3 : 1	142	28	32	32
253	3 : 1	239	65	58	54
258	3 : 1	79	19	22	14
260	3 : 1	109	15	22	20
269	3 : 1	87	22	27	16
271	3 : 1	79	19	23	15
272	3 : 1	77	15	18	11
Total		1020	237	259	
242	9 : 7	143	92	36	40
245	9 : 7	112	75	36	36
246	9 : 7	60	45	15	22
247	9 : 7	71	52	25	25
250	9 : 7	77	46	16	18
252	9 : 7	67	48	17	26
256	9 : 7	74	26	17	18
Total		604	384	162	185
248	27 : 37	36	29	6	15
261	27 : 37	92	113	20	43
268	27 : 37	76	70	11	40
273	27 : 37	90	114	27	43
Total		294	326	64	141

TABLE 12

Individual F_2 seedling distributions with respect to Y endosperm and W_2 and W_3 chlorophyll factors showing no linkage. Cross No 3.

F_1 EAR NUMBER	CHLOROPHYLL RATIO	FROM YELLOW SEEDS		FROM WHITE SEEDS		DEV. / P. E.
		Green	Albino	Green	Albino	
240	3 : 1	128	48	43	17	
254	3 : 1	114	26	42	12	
259	3 : 1	103	32	34	10	
262	3 : 1	120	41	44	14	
266	3 : 1	83	31	28	10	
267	3 : 1	59	22	29	7	
Total		607	200	220	70	0.8*
Expected		*617*	*205*	*205*	*69*	
243	9 : 7	88	90	35	28	
244	9 : 7	126	105	59	34	
251	9 : 7	106	84	34	26	
255	9 : 7	56	42	23	11	
263	9 : 7	151	102	28	32	
265	9 : 7	191	123	38	43	
270	9 : 7	67	42	35	22	
Total		785	588	252	196	1.0†
Expected		*768*	*598*	*256*	*199*	

* Calculated on basis of 10 : 6 ratio.
† Calculated on basis of 34 : 30 ratio.

TABLE 13

Individual F_2 seedling distributions with respect to aleurone and chlorophyll factors showing linkage between factors R and W_3. Cross No. 3.

F_1 EAR NUMBER	CHLOROPHYLL RATIO	FROM PURPLE SEEDS		FROM COLORLESS SEEDS	
		Green	Albino	Green	Albino
210	3 : 1	221	22	40	59
224	3 : 1	185	24	16	37
232	3 : 1	242	28	25	61
Total		648	74	81	157
202	9 : 7	105	36	16	39
203	9 : 7	93	33	11	29
204	9 : 7	105	43	11	48
206	9 : 7	121	80	15	46
208	9 : 7	144	53	15	60

TABLE 13 (continued)

F_3 EAR NUMBER	CHLOROPHYLL RATIO	FROM PURPLE SEEDS		FROM COLORLESS SEEDS	
		Green	Albino	Green	Albino
212	9 : 7	132	60	16	43
214	9 : 7	110	40	13	36
221	9 : 7	114	39	15	44
235	9 : 7	153	72	20	62
236	9 : 7	202	82	14	83
Total		1279	543	146	490
209	27 : 37	80	80	10	55
213	27 : 37	69	79	5	44
215	27 : 37	61	54	6	26
216	27 : 37	60	46	6	33
219	27 : 37	118	115	9	69
230	27 : 37	112	111	21	52
237	27 : 37	169	124	10	67
Total		669	609	67	346

TABLE 14

Individual F_2 seedling distributions with respect to R aleurone and chlorophyll factors showing no linkage. Cross No. 3.

F_3 EAR NUMBER	CHLOROPHYLL RATIO	FROM PURPLE SEEDS		FROM COLORLESS SEEDS		DEV. / P. E.
		Green	Albino	Green	Albino	
205	3 : 1	128	42	40	11	
207	3 : 1	114	37	45	15	
211	3 : 1	98	23	29	5	
218	3 : 1	69	29	31	14	
223	3 : 1	90	27	30	8	
226	3 : 1	95	26	31	6	
228	3 : 1	150	44	36	18	
229	3 : 1	85	23	31	9	
Total		829	251	273	86	1.3*
Expected		*809*	*270*	*270*	*90*	
220	9 : 7	74	43	25	20	
222	9 : 7	106	138	56	36	
231	9 : 7	115	82	59	31	
233	9 : 7	89	64	25	29	
Total		384	327	165	116	2.5†
Expected		*418*	*325*	*140*	*109*	

* Calculated from 10 : 6 ratio.

† Calculated from 34 : 30 ratio.

CROSSOVER MODIFIERS IN THE THIRD CHROMOSOME OF *DROSOPHILA MELANOGASTER*[1]

F. PAYNE
Zoölogical Laboratory, Indiana University, Bloomington, Indiana

Received March 29, 1924

In January 1916 I began an experiment to increase the number of bristles on the scutellum of *Drosophila melanogaster* by means of artificial selection. This experiment was carried through 38 generations. At the end of selection the mean bristle number had been increased from four to nine plus. In analyzing these results the strain was crossed to the multiple recessive stock, sepia, spineless, kidney, sooty, rough (all third-chromosome characters), and the F_1 flies inbred. Instead of the standard values, only 17 crossovers occurred among 3327 flies. Fifteen of these were between sepia and spineless and two were double crossovers between sepia spineless and spineless kidney (see PAYNE 1918, table 8). Of the 3327 flies 2701 were wild-type and 609 were s_e s_s k e^s r_o, a ratio of 4.4 to 1. These linkage results were explained by assuming that a crossover modifier was present in the third chromosome of the selected strain. The results show that the strain was probably homozygous for this factor and that the flies used were free from lethals. BRIDGES called this factor C_{IIIP}.

The present paper is not an analysis of the selected strain, but of lance, a second-chromosome recessive mutant which appeared December 5, 1916, within the selected strain. The term "lance" refers to the shape of the wing. In most of the females the wings are pointed instead of rounded at the tips. The character is very variable, however, and the males show it less marked than the females. In fact, the males are difficult to distinguish from the wild-type males. See figure 1 for some of the variations in wing shape and for a comparison with the wild-type. Lance has been maintained in mass cultures since its appearance, and at the present time is not the same with respect to these crossover modifiers as the selected line at the end of selection. In one third chromosome there are now two modifiers, l C_{IIIPL} and l C_{IIIPR}, which cut out nearly all crossing over in the third chromosome. The nomenclature here is slightly cumbersome, but an explanation will make it clear. C_{III} locates the crossover-modifying genes in the third chromosome; P refers to the author; while L and R place the two genes in the left and right ends of the chromosome, respectively;

[1] Contribution No. 199 from the Zöological Laboratory of INDIANA UNIVERSITY.

l means that a lethal gene is present in the vicinity of each crossover modifier and remains closely associated because of the suppression of crossing over. The other third chromosome of lance may carry a crossover modifier. At least, the modified ratios may be explained in this way; or, as we shall see later, they may be explained by assuming the modifier to be in the "IIIple" strain. The effect on crossing over is not very pronounced, however, except between roughoid and hairy, where the standard percentage is lowered from 26.0 to 18.9. There is a lethal in this chromosome which balances the lethals associated with C_{IIIPL} and C_{IIIPR}. The evidence for this lethal is found in the fact that of several hundred lance flies which have been out-crossed, all have been found to be heterozygous for $l\ C_{IIIPL}$ and $l\ C_{IIIPR}$. If this balancing lethal were not present, some

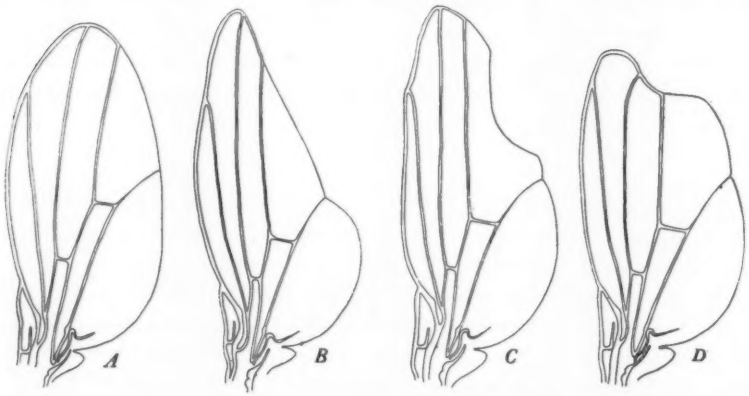


FIGURE 1.—A, wing of wild-type female. B, typical lance-shaped wing of female of lance strain. C and D, two of the variations in wing shape in the lance strain.

of the lance flies would be free from $l\ C_{IIIPL}$ and $l\ C_{IIIPR}$. It would seem that C_{IIIP} in the selected lines was the same as $l\ C_{IIIPL}\ l\ C_{IIIPR}$ without the lethals. Because of the ratio of 4.4 wild-type flies to 1 $s_e\ s_e\ k\ e^s\ r_o$, when the selected line was crossed to $s_e\ s_e\ k\ e^s\ r_o$, and the F_1 flies inbred, C_{IIIP} was known not to be lethal, and probably did not carry an associated lethal. If there had been a lethal we would have expected a 2 : 1 ratio. Further, the flies used from the selected line must have been homozygous for C_{IIIP}. The balancing lethal in the other third chromosome of lance must have been acquired since its separation from the selected line. The selected strain was started from a single pair of parents taken at Bloomington. Since both the wild-type strain from which the selected strain was started and a second strain taken in Bloomington in 1923, carried the same or similar

crossover modifiers, it seems probable that they were widely or uniformly present in the parental wild strain.

Other crossover modifiers have already been described in Drosophila. STURTEVANT (1913) was the first to discover them. He reported C_{III} at that time, though without analysis or description. This same factor, or another having the same effect and allelomorphic to it, has since been found by MULLER (1916) in spread and beaded, and by BRIDGES (1923) in maroon dwarf and in eosin. C_{III}, when heterozygous, shows its greatest inhibiting effect in the region of sooty. Between e^s and r_o no crossing over occurs; between s_s and e^s, 0.2 percent occurs. Crossing over between spineless and the left end of the chromosome approaches standard values. When C_{III} is homozygous, approximately standard values obtain in both ends of the chromosome.

BRIDGES and MORGAN (1923) report another modifier, C_{IIIM}, in the third chromosome. This was present in a wild stock from Camp Jackson, South Carolina. When heterozygous, C_{IIIM} gives the following crossover values: s_e-s_s, 28.1; s_s-e^s, 0.0; e^s-r_o, 0.5; r_o-M, 0.5. These values are very close to those for C_{III}. No tests were made for homozygous C_{IIIM}, nor to see whether C_{IIIM} might be allelomorphic to C_{III}.

BRIDGES and MORGAN (1923) also mention the presence of a modifier in the region of hairy, which increases crossing over in the hairy-scarlet section. Based upon data collected since this publication, BRIDGES writes me that this value may reach 22 $\pm$. He calls this modifier C_{III}-s_t, because it seems to be carried usually with scarlet. He also thinks there may be a slight reducer (C_{III-S}) in the left end of the third chromosome of "IIIple," and that what he has been calling "standard" may be an interaction of the two.

In the second chromosome several modifiers have been reported. The first was by STURTEVANT (1919). From a stock collected in Nova Scotia he found two modifiers, one in each end of the second chromosome. Each gene, in females heterozygous for it, cuts out nearly all crossing over in the region in which it lies. C_{IIL} is located to the left of purple and reduces the *S-b* section to 0.5. C_{IIR} is located between purple and speck. It reduces the p_r-c section to 1.6 and the c-s_p section to 0.0. Homozygous C_{IIR} gives standard crossover values. In this same stock, STURTEVANT also reports a modifier, $C_{III,\ II}$ which lies in the third chromosome, but which, in the presence of C_{IIR}, increases crossing over in the p_r-c section. This same gene, when heterozygous, also reduces crossing over in the third chromosome. Its effect is similar to, but not identical with, that of C_{III}.

WARD (1923) in a stock of curly-wing flies finds two dominant second-chromosome modifiers, C_{CL} and C_{CR}, which inhibit all crossing over along the entire length of the second chromosome under normal temperature conditions. When the flies are subjected to a higher temperature (30°C ±) crossing over frequently takes place in the right half. WARD also believes these modifiers may affect crossing over in the first and third chromosomes. Her data here, however, are far from convincing. Curly is homozygous for these modifiers.

In order to analyze the peculiar results in lance, I have made use primarily of two groups of third-chromosome linked genes, roughoid, hairy, scarlet, peach, spineless, sooty, called "IIIple," and sooty, rough, claret. For these stocks and others similar to them I am indebted to the Genetical Laboratory of COLUMBIA UNIVERSITY. I also used at times a stock made up by Doctor MULLER in which $l\,C_{IIIPL}$ and $l\,C_{IIIPR}$ were balanced against $r_u\ h\ s.\ p^p\ s_s\ H\ e^s$. I am indebted to Doctor C. B. BRIDGES for helpful suggestions and criticisms.

When lance is crossed to $r_u\ h\ s_t\ p^p\ s_s\ e^s$, and the F_1 females are back-crossed to $r_u\ h\ s_t\ p^p\ s^s\ e^s$ males, about one-half of the females show very little crossing over, while the other half show percentages slightly reduced from standard, except the region between r_u and h, which is reduced more than the others, from 26.0 to 18.9. These two classes of females have been studied separately. The complete data for every pair of parents are not given to save expense of printing. Instead, a summary of the data for each particular kind of cross is given.

TABLE 1

P_1, ♀ *lance* × ♂ *"IIIple"*; F_1 ♀ $\frac{l\,C_{IIIPL}\ l\,C_{IIIPR}}{r_u\ h\ s_t\ p^p\ s_s\ e^s}$ × ♂ *"IIIple."*

Back-cross types	r_u h s_t p^p s_s e^s	+	r_u	r_u h	r_u h s_t	p^p s_s e^s	r_u h s_t p^p s_s e^s		e^s	h s_t p^p	r_u h s_t s_s e^s	r_u h s_t	r_u h s_t p^p e^s	r_u s_t	h s_t p^p e^s
Totals	12,261	15,151	1	2	26	34	19	43	1	1	1	4	4	1	1

TABLE 2

Recombination percents computed from table 1.

Crossing-over section	r_u-h	h-s_t	s_t-p^p	p^p-s_s	s_s-e^s
Total recombinations	4	3	66	69	10
Percent of recombination	0.014	0.010	0.239	0.250	0.036
Standard percents[2]	26.0	16.4	4.2	10.2	12.2

Using the F_1 females in which little crossing over occurs we get the results shown in tables 1 and 2. Table 1 shows the different classes of individuals and the number in each class. Table 2 gives the recombination percentages for the component sections of $r_u\ h\ s_t\ p^p\ s_s\ e^s$ computed from table 1. These are as follows: r_u-h, 0.014; h-s_t, 0.010; s_t-p^p, 0.239; p^p-s_s 0.250; and s_s-e^s, 0.036. While nearly all crossing over is cut out, the greatest reduction is between r_u and h, and h and s_t. This is probably the locus of $l\,C_{\text{III PL}}$. Sooty lies at 70.7. In order to test the extreme right end of the third chromosome, tests similar to those just recorded were made with sooty, rough, claret. A lance female was crossed to an $e^s\ r_o\ c_a$ male and the F_1 females were back-crossed to $e^s\ r_o\ c_a$ males. The results for the little-crossover class are given in table 3. No crossing over occurred

TABLE 3

P_1, ♀ *lance* × ♂ $e^s\ r_o\ c_a$; F_1 ♀ $\dfrac{l\,C_{\text{III PL}}\ l\,C_{\text{III PR}}}{e^s\ r_o\ c_a}$ × ♂ $e^s\ r_o\ c_a$.

$e^s\ r_o\ c_a$	+	$e^s\ r_o$	c_a	e^s-r_o Re.[3]	e^s-r_o Sd.	r_o-c_a Re.	r_o-c_a Sd.
570	695	3	7	0.0	20.2	0.78	9.6

between e^s and r_o, and the r_o-c_a section was cut from 9.6 to 0.78. The influence of these two factors, then, extends from end to end of the third chromosome. In the right end the region of greatest reduction is between e^s and r_o, where we get no crossing over. This is probably the locus for $l\,C_{\text{III PR}}$. It is interesting in this connection that C_{III} and $C_{\text{III M}}$ also exert their greatest restriction between e^s and r_o.

In comparison with the standard percentages the section between s_t and p^p is the least affected. The standard value of 4.2 is reduced to

[2] These and subsequent standard percentages of recombination for chromosome III were obtained from the curves of figures 1 and 2, pages 12 and 13 of BRIDGES and MORGAN (1923).

[3] Wherever the abbreviations Re. and Sd. occur they refer to the recombination percent observed in the experiment and to the standard percent of recombination.

0.239. A higher percentage, 0.250, of recombination actually occurs for p^p and s_s, but the standard value here is 10.2. So, relatively, there is less effect of the crossover modifiers between s_t and p^p. BRIDGES and MORGAN (1923) point out that the effects of C_{III} and C_{IIIM} grow less in the region of peach. They believe this region to be the middle of the chromosome. Because of the lethal effect it has been impossible to get a strain of flies homozygous for C_{IIIPL} and C_{IIIPR}. This was attempted for many generations by mating wild-type flies to wild-type flies obtained in the F_2 generation from lance × "IIIple."

As crossing over occurs, occasionally, it has been possible to separate $l\,C_{IIIPL}$ from $l\,C_{IIIPR}$ and to study their effects, separately. When lance or $\frac{l\,C_{IIIPL}\;l\,C_{IIIPR}}{r_u\;h\;s_t\;p^p\;s_s\;H\;e^s}$ is mated to "IIIple" it is possible, because of a small amount of crossing over, to get F_2 flies of the following constitution: $\frac{l\,C_{IIIPL}\;p^p\;s_s\;e^s}{r_u\;h\;s_t\;p^p\;s_s\;e^s}$. If we take a male of this sort, which will be $p^p\;s_s\;e^s$ in external appearance, and mate to a wild-type stock female, by a further back-cross of the F_1 female to a "IIIple" male we can study the effect of $l\,C_{IIIPL}$ on crossing over between p^p and s_s and between s_s and e^s. The results of such a cross are summarized in table 4, which gives the actual

TABLE 4

P_1, ♂ $\frac{l\,C_{IIIPL}\;p^p\;s_s\;e^s}{\text{"IIIple"}}$ × ♀ +; F_1 ♀ $\frac{l\,C_{IIIPL}\;p^p\;s_s\;e^s}{}$ × ♂ *"IIIple."*

$p^p\;s_s\;e^s$ +		$p^p\quad s_s\;e^s$		$p^p\;s_s\quad e^s$		$p^p\;e^s\quad s^s$		p^p-s_s Re.	p^p-s_s Sd.	s_s-e^s Re.	s_s-e^s Sd.
542	682	113	103	115	107	4	5	13.4	10.2	13.8	12.2

number of individuals and the percentages of recombination. For the p^p-s_s section we obtained a recombination percent of 13.4, contrasted with standard 10.2; for s_s-e^s, 13.8 contrasted with 12.2. For both sections the recombination percents are higher than the standard values. This same type of cross has been made a second time with recombination percents of 13.9 and 14.8. Here again the values are higher than standard. Whether the differences are really significant I am unable to say.

A cross similar to that just recorded was made with an F_2 female of the following constitution: $\frac{l\,C_{IIIPL}\;s_s\;e^s}{r_u\;h\;s_t\;|\;p^p\;s_s\;e^s}$. She was mated to a wild-type male from stock and the females from this cross mated back to "IIIple" males. The offspring of the $\frac{l\,C_{IIIPL}\;s_s\;e^s}{}$ females only were counted and tabulated in table 5. The recombination percent for the s_s-e^s section was 10.3, slightly less than the standard value of 12.2. The numbers given

in table 5 are the sums of two separate experiments performed at widely different times. In one the value for s_s-e^s was 10.2 and other 10.3. If the increased values in table 4 are significant, it would seem strange that we

TABLE 5

P_1, ♀ $\frac{l\ C_{\text{IIIPL}}\ s_s\ e^s}{r_u\ h\ s_t\ p^p\ s_s\ e^s}$ × ♂ +; F_1 ♀ $\frac{l\ C_{\text{IIIPL}}\ s_s\ e^s}{}$ × ♂ *"IIIple."*

$s_s\ e^s$	+	s_s	e^s	s_s-e^s Re.	s_s-e^s Sd.
1175	1423	142	157	10.3	12.2

should get a reduced value here. Certainly $l\ C_{\text{IIIPL}}$ is present in both cases. The only difference is in the absence or presence of the region of the chromosome including p^p. Pink, spineless, sooty flies of the following constitution, $\frac{l\ C_{\text{IIIPL}}\ p^p\ s_s\ e^s}{r_u\ h\ s_t\ p^p\ s_s\ e}$, have been mated *inter se*, in an attempt to produce flies homozygous for the crossover modifier. The result of such a cross was 370 $p^p\ s_s\ e^s$ flies to 102 $r_u\ h\ s_t\ p^p\ s_s\ e^s$. The $p^p\ s_s\ e^s$ flies were not homozygous, however. When inbred they again threw $p^p\ s_s\ e^s$ and $r_u\ h\ s_t\ p^p\ s_s\ e^s$ flies. This clearly demonstrates the presence of a lethal associated with the crossover modifier.

I also crossed F_2 $\frac{r_u\ h\ s_t\ l\ C_{\text{IIIPR}}}{r_u\ h\ s_t\ p^p\ s_s\ e^s}$ males to wild-type females from stock and back-crossed the females to "IIIple" males. The offspring of the

TABLE 6

P_1 ♂ $\frac{r_u\ h\ s_t\ l\ C_{\text{IIIPR}}}{r_u\ h\ s_t\ p^p\ s_s\ e^s}$ × ♀ +; F_1 ♀ $\frac{r_u\ h\ s_t\ l\ C_{\text{IIIPR}}}{}$ × ♂ *"IIIple."*

$r_u\ h\ s_t$	+	r_u	$h\ s_t$	$r_u\ h$	s_t	$r_u\ s_t$	h	r_u-h Re.	r_u-h Sd.	h-s_t Re.	h-s_t Sd.
689	805	333	281	294	340	27	23	23.8	26.0	24.5	16.4

$\frac{r_u\ h\ s_t\ l\ C_{\text{IIIPR}}}{}$ females have been tabulated in table 6, which gives the numbers of individuals and the percentages of recombination. Between r_u and h I found 23.8 and between h and s_t, 24.5 percent. The value for the roughoid-hairy section is slightly low but probably not significantly so. The value for the hairy-scarlet section is very much increased, from 16.4 to 24.5. This is certainly a significant difference. It has been obtained in separate experiments. It would seem, then, that $l\ C_{\text{IIIPR}}$ in the absence of $l\ C_{\text{IIIPL}}$ increases crossing over between hairy and scarlet. It is probable, however, that the modifier is in the "IIIple" strain, and that it is the same as, or similar in its action to, C_{III}-s_t.

A similar cross was made with a $\frac{r_u\ h\ s_t\ p^p\ l\ C_{\text{III PR}}}{r_u\ h\ s_t\ p^p\ s_e\ e^s}$ male. The only difference here is the addition of p^p to the chromosome with $l\ C_{\text{III PR}}$. The results are given in table 7. Here we get practically standard values for

TABLE 7

P_1 ♂ $\frac{r_u\ h\ s_t\ p^p\ l\ C_{\text{III PR}}}{\text{"}III ple\text{"}}$ × ♀ +; F_1 ♀ $\frac{r_u\ h\ s_t\ p^p\ l\ C_{\text{III PR}}}{\text{"}III ple\text{"}}$ × ♂ *"IIIple."*

r_u h s_t p^p	+	r_u	h s_t p^p	r_u h	s_t p^p	r_u h s_t	p^p	r_u s_t p^p	h	r_u p^p	h s_t	r_u h p^p	s_t	r_u h Re.	r_u h Sd.	h s_t Re.	h s_t Sd.	s_t p^p Re.	s_t p^p Sd.
463	514	210	186	227	226	36	35	26	26	6	13	2	3	26.7	26.0	25.8	16.4	4.8	4.2

the r_u-h and s_t-p^p sections, 26.7 against 26.0, and 4.8 against 4.2. On the other hand, we get the increased value for the h-s_t section, 25.8 against 16.4. This experiment also shows that the inhibiting effect of $l\ C_{\text{III PR}}$ does not extend to the left of pink. $\frac{r_u\ h\ s_t\ l\ C_{\text{III PR}}}{r_u\ h\ s_t\ p^p\ s_e\ e^s}$ flies have been inbred in an attempt to produce homozygous $l\ C_{\text{III PR}}$ flies. The result is to produce flies of the same heterozygous constitution. The strain cannot be made homozygous for $l\ C_{\text{III PR}}$.

To test the further effects of these modifiers, $\frac{r_u\ h\ s_t\ l\ C_{\text{III PR}}}{r_u\ h\ s_t\ p^p\ s_e\ e^s}$ flies were crossed to $\frac{l\ C_{\text{III PL}}\ p^p\ s_e\ e^s}{r_u\ h\ s_t\ p^p\ s_e\ e^s}$ flies. The result was four kinds of flies: wild-type 227, $r_u\ h\ s_t$ 166, $p^p\ s_e\ e^s$ 194, and $r_u\ h\ s_t\ p^p\ s_e\ e^s$ 162. This is what we would expect from previous results.

One third chromosome then, contains two crossover modifiers which are associated with lethals. One of these genes, $l\ C_{\text{III PL}}$ lies in the left end in the region of hairy and restricts crossing over in this end as far as peach and perhaps for a few units to the right of peach. The other modifier, $l\ C_{\text{III PR}}$, lies in the right half of the chromosome in the region of sooty. It restricts crossing over in this end as far to the left as spineless and perhaps for some units beyond spineless. The increased crossing over between roughoid and hairy is perhaps best explained by assuming that the modifier lies in the third chromosome of "IIIple."

As previously stated, the two third chromosomes of lance are not alike. The one which we have just analyzed cuts out nearly all crossing over. When lance is crossed to "IIIple," half of the F_1 females will have the third chromosomes as follows: $\frac{l}{r_u\ h\ s_t\ p^p\ s_e\ e^s}$. When these females are

back-crossed to "IIIple" males, crossing over between all the genes is reduced a small amount, but the section between roughoid and hairy is reduced more than the others, from 26.0 to 18.9 (tables 8 and 9). This

TABLE 8

*P*₁, ♀ *lance*×♂ "*IIIple*"; *F*₁ ♀ $\frac{l}{r_u\ h\ s_t\ p^p\ s_s\ e^s}$×♂ "*IIIple*."

Control, ♀ "*Texas wild*"×♂ "*IIIple*"; *F*₁ ♀×"*IIIple*" ♂.

Back-cross types	r_u h s_t p^p s_s e^s	+	r_u h s_t p^p s_s e^s		r_u h s_t p^p s_s e^s		r_u h s_t p^p s_s e^s		r_u h s_t p^p s_s e^s		r_u h s_t p^p s_s e^s		r_u h s_t p^p s_s e^s		r_u h s_t p^p s_s e^s		r_u h s_t p^p s_s e^s		r_u h s_t p^p s_s e^s	
l	809	1031	284	249	249	206	20	24	54	94	28	134[4]	1	4	1		26	13	44	12
Control	767	928	353	280	225	207	28	20	74	66	42	117	6	12	4	7	26	18	38	35

TABLE 8 (continued)

r_u h p^p s_s e^s	s_t	r_u h s_t p^p s_s e^s		r_u h s_t p^p s_s e^s		r_u h s_t p^p s_s e^s		r_u h s_t p^p s_s e^s		r_u h s_t p^p s_s e^s		h p^p s_s e^s		r_u h s_t p^p s_s e^s		r_u h s_t p^p s_s e^s		h s_t		r_u h s_t p^p s_s e^s		r_u h s_t p^p s_s e^s		s_t p^p e^s		s_t p^p e^s	
	2	13	20	33	6		1	1	1	5			2			1			2	1			1		1	
2	2	21	31	24	15	6	5	1	5	3	2	1	3	1	1	3	4			1	3		1		1	

TABLE 9

Recombination percents computed from table 8.

Crossing-over sections	r_u-h	h-s_t	s_t-p^p	p^p-s_s	s_s-e^s
Total recombinations, *l*	639	539	51	232	270
Percent of recombinations, *l*	18.9	15.9	1.5	6.8	8.0
Total recombinations, "control"	793	558	109	261	293
Percent of recombinations, "control"	23.4	16.4	3.2	7.7	8.6
Standard percentages	26.0	16.4	4.2	10.3	12.2

type of cross has been made five different times, with results very similar to those in tables 8 and 9. I have been rather reluctant to admit that the

[4] At times difficulty has been encountered in the identification of sooty, hence some errors are probably present in the recombination values for the s_s-e^s section. Since I have drawn no conclusions concerning this section the errors in no way obviate my general results. It is possible that sooty intensifiers have caused the trouble.

reductions, except that between roughoid and hairy, were significant, and I am not at all sure that they are. Another reason why I am somewhat skeptical as to these reduced values is that I have crossed "IIIple" to a wild-type strain from Texas and back-crossed the F_1 females to "IIIple" males. This ought to give standard values. The results are given in table 8 as a control. All values are low. It is true, they are slightly higher than those in the cross with lance, but the differences are not large except in the case of the roughoid-hairy section. In the control this value approaches the standard.

Lance was also crossed to $e^s\ r_o\ c_a$ and the F_1 $\frac{l}{e^s\ r_o\ c_a}$ females back-crossed to $e^s\ r_o\ c_a$ males. The results are given in table 10. The recombination values are 17.8 and 10.2, against standard values of 20.2 and 9.6. The e^s-r_o section is slightly low, the r_o-c_a section slightly high. It would seem there were no effects of a modifier upon the extreme right end.

TABLE 10

P_1, ♀ $e^s\ r_o\ c_a \times$ ♂ *lance*; F_1 ♀ $\frac{e^s\ r_o\ c_a}{l} \times$ ♂ $e^s\ r_o\ c_a$.

$e^s\ r_o\ c_a$	+	e^s	$r_o c_a$	$e^s\ r_o$	c_a	$e^s c_a$	r_o	e^s-r_o Re.	e^s-r_o Sd.	r_o-c_a Re.	r_o-c_a Sd.
508	580	136	131	77	75	2	0	17.8	20.2	10.2	9.6

In a further study of this same third chromosome, lance was mated to "IIIple" and the F_1 females back-crossed to "IIIple" males. Some of the F_1 females show crossing over. From this group F_2 males were selected with different sections of the third chromosome from lance. When an F_2 $\frac{r_u\ h\ s_t\ \ ?}{r_u\ h\ s_t\ p^p\ s_e\ e^s}$ male is crossed out to a wild-type female there will be two types of individuals produced, since there is no crossing over in the male. One type will be $\frac{r_u\ h\ s_t\ \ ?}{\qquad\qquad}$, and it is this type which we mated back to "IIIple" males. The left end of one third chromosome comes from "IIIple," the right end from lance. The other third chromosome comes from the wild stock. This experiment gives a reduced value of 19.8 for the r_u-h section (similar to that of table 8), and an increased value of 21.2 for the h-s_t section (table 11). This h-s_t value is similar to that obtained with $l\ C_{\text{IIIPR}}$ in the absence of $l\ C_{\text{IIIPL}}$ (tables 6 and 7).

When we use the left end of this same chromosome combined with $p^p\ s_e\ e^s$ and $s_t\ p^p\ s_e\ e^s$ we get approximately standard values for p^p-s_e and

TABLE 11

P_1, ♂ $\frac{r_u\ h\ s_t \quad ?}{r_u\ h\ s_t\ p^p\ s_a\ e^s}$ × ♀ +; F_1 ♀ $\frac{r_u\ h\ s_t \quad ?}{\qquad\qquad}$ × ♂ *"IIIple."*

$r_u\ h\ s_t$	+	r_u	$h\ s_t$	$r_u\ h$	s_t	$r_u\ s_t$	h	r_u-h Re.	r_u-h Sd.	h-s_t Re.	h-s_t Sd.
338	478	114	137	140	129	9	8	19.8	26.0	21.1	16.4

s_a-e^s (tables 12 and 13). The s_t-p^p region is reduced, but again it is doubtful whether the reduction is significant.

TABLE 12

P_1, ♀ $\frac{?\quad p^p\ s_a\ e^s}{r_u\ h\ s_t\ p^p\ s_a\ e^s}$ × ♀ +; F_1 ♀ $\frac{?\quad p^p\ s_a\ e^s}{\qquad\qquad}$ × ♂ *"IIIple."*

$p^p s_a\ e^s$	+	p^p	$s_a\ e^s$	$p^p\ s_a$	e^s	$p^p\ e^s$	s_a	p^p-s_a Re.	p^p-s_a Sd.	s_a-e^s Re.	s_a-e^s Sd.
348	439	60	42	49	61	1	1	10.3	10.3	11.1	12.2

TABLE 13

P_1, ♂ $\frac{?\ s_t\ p^p\ s_a\ e^s}{r_u\ h\ s_t\ p^p\ s_a\ e^s}$ × ♀ +; F_1 ♀ $\frac{?\ s_t\ p^p\ s_a\ e^s}{\qquad\qquad}$ × ♂ *"IIIple."*

s_t p^p s_a e^s	+	s_t	p^p s_a e^s	s_t p^p	s_t p^p s_a e^s	s_t p^p s_a	s_t p^p e^s	s_t s_a e^s	p^p	p^p s_a	s_t p^p Re.	s_t p^p Sd.	p^p s_a Re.	p^p s_a Sd.	s_a e^s Re.	s_a e^s Sd.
647	831	19	12	126	128	124	168	1	15	1	1	2	1.6	4.2	13.1	10.3	14.9	12.2

As indicated in tables 6 and 7, $l\,C_{\text{IIIPR}}$ in the absence of $l\,C_{\text{IIIPL}}$, seems to increase crossing over between hairy and scarlet; but, as previously stated, this result can be explained by assuming that the modifier is in the "IIIple" strain. In table 11, where we use the right end of the second third chromosome of lance, we get both a decrease in the r_u-h section and an increase in the h-s_t section. Here, then, we get in the same experiment, what seemed to be the effect of $l\,C_{\text{IIIPR}}$, and of a modifier in the other third chromosome of lance. These results would seem to make it practically conclusive that the modifiers lie in the "IIIple" strain and that they are similar in their action to C_{III}-s_t and C_{IIIL}-S.

Considerable difficulty has been experienced in analyzing these crossover modifiers, due to the fact that two wild-type stocks used in outcrosses, and taken in Bloomington at widely different times, have either

the same crossover factors or others which have about the same influence. These strains were not the same as lance with respect to these factors, for they give different results when crossed to "IIIple". When lance is crossed to "IIIple" and the F_1 females back-crossed to "IIIple" males, we find there are two types of F_1 females. In one type very little crossing over occurs; in the other, crossing over approaches standard values. When one of these wild-type strains, called B1, is crossed to "IIIple" and the F_1 females back-crossed to "IIIple" males, we again find two types of F_1 females, but they are not the same as in the lance cross. In one type we find very little crossing over in the left end (tables 14 and 15); in the second type very little crossing over in the right end (tables 14 and 15). Evidently the crossover modifiers are in the wild strain and the probability is that we have C_{IIIPL} in one and C_{IIIPR} in the other.

When the second wild-type strain, B2, was crossed to "IIIple" and the F_1 females back-crossed to "IIIple" males, we found there were three kinds of F_1 females. In one group very little crossing over occurs (tables 14 and 15). These results are similar to those obtained when $l\,C_{IIIPL}$ and $l\,C_{IIIPR}$ are present. In the second group crossing over is restricted in the right end and increased between hairy and scarlet (tables 14 and 15). The results are similar to those obtained with $l\,C_{IIIPR}$. In the third group crossover values approach the standard, except possibly between roughoid and hairy, where it is reduced by 4.5 percent (tables 14 and 15).

TABLE 14

+ ♀ (*B1 wild-type stock*) × ♂ "*IIIple*"; F_1 ♀ × ♂ "*IIIple.*"
+ ♀ (*B2 wild-type stock*) × ♂ "*IIIple*"; F_1 ♀ × ♂ "*IIIple.*"

Back-cross types		+																	
		r_u h s_t p^p s_s e^s		r_u h s_t p^p s_s e^s		r_u h s_t p^p s_s e^s		r_u h s_t p^p s_s e^s		r_u h s_t p^p s_s e^s		r_u h s_t p^p s_s e^s		r_u h s_t p^p s_s e^s		r_u h s_t p^p s_s e	
B1, CL		178	180					2	1	9	30		41						
B1,	CR	331	538	147	189	110	123	11	12		1			8	12	6	6		
B2, CL	CR	652	888					2	2	1	7								
B2,	CR	533	657	261	195	233	220	37	43	9	12			8	10	12	8		
B2,		484	470	146	178	123	138	20	17	49	58	40	76	7	11	3	2		

TABLE 14 (continued)

r_u	h	r_u	h	r_u h	s_t	r_u h	s_t	r_u h	s_t	r_u h	s_t	r_u h	s_t	r_u h	s_t	r_u h	s_t	h
	p^p		p^p	p^p		p^p		p^p		p^p		p^p		p^p		p^p		
s_s		s_s		s_s		s_s		s_s		s_s		s_s		s_s		s_s		
e^e		e^e		e^e		e^e		e^e		e^e		e^e		e^e		e^e		e^e
				1						2		4						
				3	1													
6	7	6		8	11	3	8	1	6	2	2	8	1	2				
19	18	20	14	3	3	14	13	24	16	2	2	2	3	7	2	1	1	1

TABLE 14 (continued)

r_u	h	r_u	h	h	h	r_u h	h	r_u	r_u			r_u	h
	s_t	s_t		s_t	s_t		s_t			s_t		s_t	
p^p			p^p	p^p		p^p		p^p		p^p	p^p	p^p	p^p
s_s			s_s		s_s				s_s				
	e^e		e^e	e^e	e^e		e^e			e^e	e^e	e^e	
	1	1	1		1	1				5		1	1
1	2		2	1			1	1		1	1		1

TABLE 15

Recombination values computed from table 14.

	r_u-h	h-s_t	s_t-p^p	p^p s_s	s_s-e^e
Crossing-over sections					
Total recombinations, B1, CL	0	1	6	39	47
Percent of recombinations, B1, CL	0.0	0.2	1.3	8.7	10.4
Total recombinations, B1, CR	368	257	39	1	0
Percent of recombinations, B1, CR	24.5	17.1	2.6	0.06	0.0
Total recombinations, B2, CL CR	0	0	4	8	0
Percent of recombinations, B2, CL CR	0.0	0.0	.25	.51	0.0
Total recombinations, B2, CR	519	518	137	60	31
Percent of recombinations, B2, CR	22.3	22.3	5.9	2.5	1.3
Total recombinations, B2	434	359	64	193	212
Percent of recombinations, B2	21.7	17.9	3.2	9.6	10.6
Standard percentages	26.0	16.4	4.2	10.3	12.2

In an attempt to see whether the modifiers present in lance have any effect upon crossing over in the second chromosome, lance was mated to black, purple, curved, plexus, speck, and the F_1 females back-crossed to $b\ p_r\ c\ p_z\ s_p$ males. The results of this cross are given in table 16, cross A. The recombination values for b-p_r, p_r c, and c-s_p are 9.6, 23.5 and 25.5, against standard values of 6.0, 21.0 and 31.5. The first two values are high, the third one low. I doubt whether the differences are significant. By using Doctor MULLER's stock of $\frac{l\ C_{\text{IIIPL}}\ l\ C_{\text{IIIPR}}}{r_u\ h s_t\ p^p\ s_s\ H e^{ey}}$ it was possible to test the effects of $l\ C_{\text{IIIPL}}$ and $l\ C_{\text{IIIPR}}$. This stock was crossed to $b\ p_r\ c\ p_z\ s_p$ and the F_1 not-hairy females back-crossed to $b\ p_r\ c\ p_z\ s_p$ males. The results are given in table 16, cross B, and the percentages of recombination approach very

TABLE 16

Cross A. F_1 ♀ $\frac{l\ C_{\text{IIIPL}}\ l\ C_{\text{IIIPR}}}{b\ p_r\ c\ s_p}$, *cr* $\frac{\quad}{l}$ $\frac{b\ p_r\ c\ s_p}{\quad}$ × ♂ $b\ p_r\ c\ s_p$.

Cross B. F_1 ♀ $\frac{l\ C_{\text{IIIPL}}\ l\ C_{\text{IIIPR}}}{b\ p_r\ c\ s_p}$ × ♂ $b\ p_r\ c\ s_p$.

Back-cross types	b p_r c s_p	+	b p_r c	s_p	b p_r	c	b p_r c	s_p	b c	p_r	b p_r c	s_p	b p_r	c	s_p	b p_r Re.	b p_r Sd.	p_r c Re.	p_r c Sd.	c s_p Re.	c s_p Sd.
Cross A	425	522	63	41	199	187	195	263	3	14	26	39	43	50		7	9	9.6	6.2[5]	23.5	19.9	25.5	30.2
Cross B	466	682	39	8	113	215	225	322	7	2	20	22	50	125		15	1	4.6	6.2	22.8	19.9	33.5	30.2

closely standard values. They are 4.6 against 6.0; 22.8 against 21.0; and 33.5 against 31.5. It would seem there was certainly no influence here, and no conclusive evidence in the former case.

To test whether there were any effects upon crossing over in the first chromosome, lance was also crossed to a stock having the first-chromosome characters eosin and miniature, and the F_1 females were back-crossed to eosin miniature. The result was 31.3 percent of recombination against the standard value of 33.2 (table 17). There was no effect on this region of the first chromosome.

TABLE 17

P_1, ♀ lance × ♂ eosin miniature; F_1 ♀ × ♂ eosin miniature.

$w^e m$	+	w^e	m	$w^e m$ Re.	$w^e m$ Sd.
413	395	166	203	31.3	32.2[6]

[5] The standard percentages were taken from table 140, page 298, of BRIDGES and MORGAN (1919).

[6] This standard recombination percentage was taken from table 65, page 84, of MORGAN and BRIDGES (1916).

At the beginning it was taken for granted that the gene for lance was in the third chromosome. Tests with star dichaete, however, show it to be in the second. Lance was mated to *S D*, and the F_1 *S D* males backcrossed to lance females. The result was 286 *S D* flies, 116 *D* lance, 158 lance, and 178 *S*. No attempt was made to locate lance within the chromosome. Because of the great variability of lance and the fact that these variations when inbred do not breed true, leads me to suspect that lance may be due to more than one gene.

When lance is mated to wild-type flies from stock and the F_1 flies inbred we do not get a 3 : 1 ratio. By actual count we obtained 1923 wild-type flies to 240 lance, a ratio of 8 : 1. This extreme ratio may be due, in part, to failure to recognize all lance flies; to the poor viability of lance; or again if lance is the effect produced by several genes, to the separation of these genes.

SUMMARY

1. Lance is a second-chromosome character and highly variable. The wing is pointed.

2. One of the third chromosomes of lance carries two crossover modifiers, C_{IIIPL} and C_{IIIPR}, each associated with a lethal. *l* C_{IIIPL} lies in the region of hairy and scarlet and restricts crossing over in the left half of the chromosome. *l* C_{IIIPR} lies in the region of ebony and restricts crossing over in the right half. The high $h\text{-}s_t$ value obtained with *l* C_{IIIPR} can be best explained by assuming the presence of a modifier in the third chromosome of "IIIple."

3. The second third chromosome of lance may carry a crossover modifier which reduces the roughoid-hairy value from 26.0 to 18.9. The change is perhaps better explained by assuming the crossover modifier to be in the left end of the third chromosome of "IIIple." The reduced values in other regions are of doubtful significance. There is a lethal gene in this chromosome.

4. The modifiers produce no noticeable effect upon crossing over either in the first or second chromosomes.

5. Because the two third chromosomes carry different lethals, and also linkage modifiers that prevent these lethals from crossing over with another, the stock is maintained as a balanced lethal stock.

6. In the original stock C_{IIIPL} and C_{IIIPR} were not associated with lethals.

LITERATURE CITED

BRIDGES, C. B., 1915 A linkage variation in Drosophila. Jour. Exp. Zoö'. **19**: 1–21.

BRIDGES, C. B., and MORGAN, T. H., 1919 The second-chromosome group of mutant characters. Carnegie Inst. Washington Pub. 278, pp. 123–304.

1923 The third-chromosome group of mutant characters of *Drosophila melanogaster*. Carnegie Inst. Washington Pub. 327, 250 pp.

DETLEFSEN, J. A., 1920 Is crossing over a function of distance? Proc. Nation. Acad. Sci. **6**: 663–670.

DETLEFSEN, J. A., and ROBERTS, E., 1921 Studies on crossing over. 1. The effect of selection on crossover values. Jour. Exp. Zoöl. **32**: 333–354.

MORGAN, T. H., and BRIDGES, C. B., 1916 Sex-linked inheritance in Drosophila. Carnegie Inst. Washington Pub. 237. 87 pp.

MULLER, H. J., 1916 The mechanism of crossing over. Amer. Nat. **50**: 193–221, 284–305, 350–366, 421–434.

PAYNE, F., 1918 An experiment to test the nature of variations on which selection acts. Indiana Univ. Studies **5**: 1–45.

PLOUGH, H. H., 1917 The effect of temperature on crossing over in Drosophila. Jour. Exp. Zoöl. **24**: 147–209.

STURTEVANT, A. H., 1917 Genetic factors affecting the strength of linkage in Drosophila. Proc. Nation. Acad. Sci., **3**: 555–558.

1919 Inherited linkage variations in the second chromosome. Carnegie Inst. Washington Pub. 278. pp. 305–387.

WARD, L., 1923 The genetics of curly-wing in Drosophila, another case of balanced lethal factors. Genetics **8**: 276–300.

TWO NEW HEREDITARY TUMORS IN DROSOPHILA[1]

IRA T. WILSON
Heidelberg University, Tiffin, Ohio

Received March 29, 1924

TABLE OF CONTENTS

	PAGE
INTRODUCTION	343
Description of tumors	345
Description of tumor number 1	345
Frequency	345
Gross appearance	345
Relationship to the normal tissues	346
Growth and fate of tumor	347
Cellular structure	347
Origin	348
Mortality	348
Description of tumor number 2	348
Frequency	348
Gross appearance	348
Relationship to normal tissues	350
Growth and fate of tumor	350
Ceilular structure	350
Origin	352
Mortality	352
Discussion	352
Heredity of the tumors	352
Heredity of tumor number 1	352
Heredity of tumor number 2	355
Cross of tumor number 1 and tumor number 2	358
General statement	358
The influence of the environment	358
DISCUSSION	360
SUMMARY	361
LITERATURE CITED	361

INTRODUCTION

BRIDGES's (1916) lethal 7, which is a sex-linked factor located at point 0.1, was found by Miss STARK (1918, 1919 a) to be due to a tumor. This was the first demonstration of the nature of the cause of death by a lethal factor. It occurs in one-half of the male larvae and kills all such in the larval stage. Miss STARK found that the tumor tissue develops from

[1] Contribution No. 205 from the Zoölogical Laboratory of INDIANA UNIVERSITY.

the embryonic rudiments destined to develop the adult organs during the pupal stage and is accompanied by excessive production of melanin (pigment). She described the histological appearance of this tumor as "cellular growths somewhat resembling the tumors of vertebrates." She further states that,

"The cells near the center are large, polyhedral, spheroidal, or fusiform in shape. Pigment is present both within and outside the cells. Toward the periphery the cells become flattened and closely crowded together, forming laminated layers of pigment giving to the tumor the appearance of being encapsulated."

She demonstrated that the tumor is not due to a micro-organism. She inferred that a toxin is produced which causes the death of the larvae, because tumors, when injected into normal larvae of other strains, caused their death.

Miss STARK (1919 b) reported that another tumor had occurred as a mutation in the strain of flies containing lethal 7. It is non-lethal, hereditary and benign. It develops in the larval stage or later and is carried into the adult, but causes no serious trouble to the flies having it. She found that at least one of the essential genes is located in the third chromosome, closely linked to dichaete which is located at 40.4. BRIDGES and MORGAN (1923, p. 180) say,

"Later unpublished work of Miss STARK shows that the locus of be–III is about 15 units to the left of dichaete, or at 25±. A complementary gene is located about 10 units to the left of black; and there is probably a IV-chromosome modifier also. The inheritance is exceptionally hard to follow, and these locations are only tentative."

Miss STARK described the benign tumor thus,

"The cells are rounded or polygonal in shape and contain pigment. As the tumor grows older the amount of pigment increases, the cells filled with it become crowded towards the periphery and flattened,"

and the tumor

"Takes its origin in groups of cells similar in structure to the cells found just inside of the hypodermis of the larva. These cells are originally derived from the hypodermal cells. In the fly the tumor, when fully matured, is entirely permeated by pigment and is black in color. The cells have stopped increasing in number. As the fly grows older the tumor tends to shrink in size but does not disappear."

She found flies in which the tumor had prevented the development of a leg or wing by ingrowths of the tumor cells into the imaginal discs of those organs. The tumor occurs throughout the body, even in the head; but

causes no apparent disturbance, when occurring in the thorax or abdomen, if it does not prevent the growth of the legs or wings.

Doctor F. PAYNE turned over to me a strain of flies in which he had observed for several years tumors similar to those described by Miss STARK. This tumor I shall call tumor No. 1. Later he turned over to me a single female from an altogether different strain which had a large black spot in its abdomen, and which I mated out to a wild strain of flies and from which by selection I subsequently got a pure strain of tumor flies. This latter tumor I shall call tumor No. 2. I have worked out the gross appearance, the relation of the tumors to the larval tissues, the histology, and the genetics of these two tumors in so far as I was able. Also, in the case of the first tumor, which develops in only a few of the larvae, I was able to show that some causal relationship exists between the environment and the number of flies developing the tumor.

I am especially indebted to Doctor PAYNE, under whom I did this work, for the tumor strains of flies and for his directions and suggestions throughout the progress of the work. Doctor MARY B. STARK has kindly given me much help. I am indebted to Doctor T. H. MORGAN for mutant strains of flies which were useful in analyzing the genetic factors underlying tumor development and especially for the great care with which he went over the manuscript of this paper. I am also indebted to Doctor C. B. BRIDGES for going over the manuscript.

DESCRIPTION OF THE TUMORS

Description of tumor number 1

Frequency

In an apparently pure stock this tumor occurs in the larvae in about 20 percent of cases and in adults in about 14 percent. There is great fluctuation in the frequency from culture to culture, apparently due to environmental differences.

Gross appearance

In the larva the tumor develops as one or more black spots located at almost any point from the extreme anterior to the extreme posterior end, but most frequently in about the middle of the posterior half (figures 4 and 5, plate 1). It is usually found in the abdomen of the adult also, but infrequently is found in the thorax or head. The blackness is due to the deposit of black pigment. Very young larvae (one to two days old) are found infrequently with tumors, the majority of tumors appearing when

the larvae are about half to two-thirds grown, and a few showing tumors only in the last stages of larval life. Some tumors must develop during late pupal or early adult life because I find adults with tumors which, although examined daily up to hatching from the pupa case, showed no visible tumors. Usually adults with tumors have carried them through from the larval stage. The tumors have made a large part of their growth before pigment deposition begins. By the time a tumor has become fully

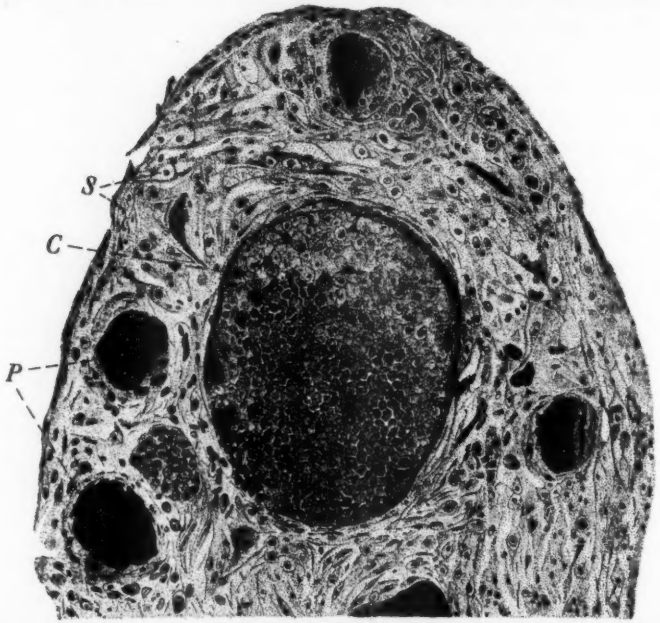


FIGURE 1.—Section of about half of isolated extra-large primary first tumor. *P*, centers of pigment deposit; *C*, center of active growth; *S*, spindle-shaped light-staining cells which form bulk of tumor and lie in homogeneous non-cellular ground substance. ×300.

pigmented the death of the larva has taken place or pupation has begun. The death of such larvae seems connected in some way with the maturity of the tumor. Sometimes as many as a hundred small tumors are scattered throughout the body.

Relationship to the normal tissues

Microscopical examination shows that the tumor tissue usually lies in the body cavity. Sometimes it is connected with the hypodermis, and at other times it lies between the folds of the intestine. Rarely it is

connected to the neuroblast or inside of the neuroblast (see figure 9, plate 1). It is found frequently to have displaced normal tissue, e.g., whole groups of muscles have been displaced by the tumor tissue (see figure 8, plate 1).

Growth and fate of tumor

When the larva lives long enough to reach the adult stage the cellular structure of this tumor and its histological appearance undergo definite changes. In a young tumor the cellular structure is quite clearly distinguishable. A little later, however, centers of pigment appear (figure 1), with large spaces remaining between the pigment centers. Soon pupation takes place, and the cellular structure is removed by histolysis, so that in the adults (figure 7, plate 1) usually little can be distinguished but pigment.

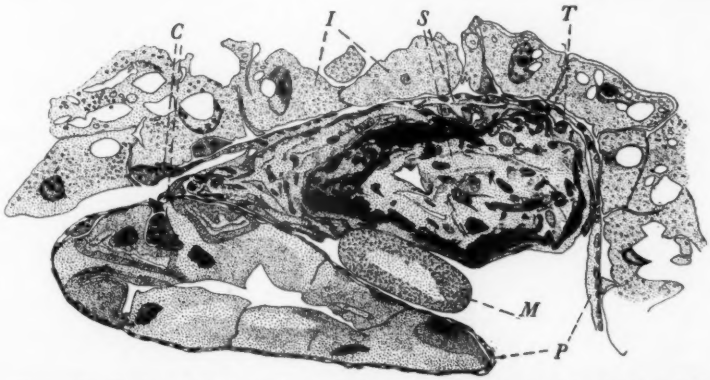


FIGURE 2.—Section of larva showing a nearly mature second tumor lying between folds of intestines. Large black broken ring is pigment deposited by the tumor cells. Cells inside and outside the black ring up to first solid line are tumor cells. Along the left-hand side of the upper edge the tumor cells seem to be continuous with peritoneal coat of the intestine. *C*, imaginal discs of adult intestine; *I*, cells of intestine; *M*, Malpighian tubule; *P*, peritoneal coat of intestine; *S*, ground work of stroma of the tumor; *T*, typical tumor cell. ×294.

Cellular structure

The deposit of pigment in centers is very characteristic of this tumor. As is seen in figure 1 there are several centers of pigment deposit, some fully developed, as at *P*, and others apparently just beginning to be deposited, as at *C*. The cells in the center shown at *C* are polygonal, probably due to crowding. At this time they stain more deeply than do

other parts of the tumor. The cells outside these denser centers are spindle-shaped and are apparently embedded in a non-cellular stroma in which can be seen fiber-like structures. At the edges of large tumors, not shown in this figure, these spindle-shaped cells can be seen to be only partially attached to the tumor, and the body cavity is filled with thousands of them which have broken loose. They may give rise to the smaller tumors in other parts. The smaller tumors have the same cellular structure as that of larger tumors.

Origin

Since I find the tumor located in, or in connection with, the hypodermis, nerve tissue, and intestine, it seems that there may be several places of origin. In one case I have sections showing graded transitions from hypodermis to tumor tissue and I have no doubt but that this tumor was derived from the hypodermis.

Mortality

The mortality of the larvae that show this tumor is very great, 65 percent of them dying before pupation, as compared with about 45 percent for those larvae, of the same strain, that did not show tumors. Furthermore, 40 percent of those that reach the pupal stage die before hatching, as compared with 22 percent of normal pupae. Stated in another way, 81 percent of the individuals in which tumors can be seen die in the larval or pupal stages as compared with 57 percent for those that do not show tumors. The higher mortality is probably due indirectly to the fairly early development of the tumor and consequently greater opportunity for growth, and to the larger number of small tumors. In some cases death may be caused by invasive growth into vital organs and by derangement of others. It may be that a toxin is produced by the tumor cells which is the direct cause of death, as Miss STARK concluded was the case for lethal 7.

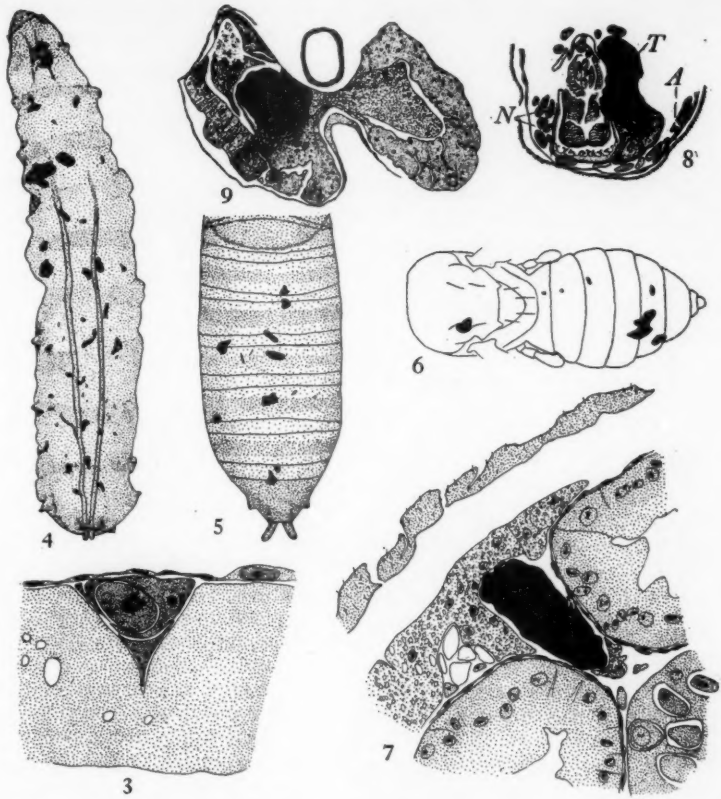
Description of tumor number 2

Frequency

This tumor shows in practically all of the larvae and adults and is much easier to study for that reason.

Gross appearance

This tumor usually appears in the latter part of the larval period of the fly, most frequently on the last day before pupation, and is first noticed



All drawings are of tumor No. 1, except figure 3.

FIGURE 3.—Part of intestinal wall showing imaginal disc of adult intestine. ×820.

FIGURE 4.—Large larva with many tumors.

FIGURES 5 and 6.—Pupa and adult with tumors carried over from larval stage.

FIGURE 7.—Section showing pigment of tumor in adult. ×147.

FIGURE 8.—Section through pharynx of a larva showing tumor (*T*) displacing group of muscles (*A*) from normal position (*N*).

FIGURE 9.—Section showing tumor lying entirely within neuroblast. ×215.

as a large black spot or only a few spots (figures 10 and 11, plate 2) located nearly always in the posterior part of the larva. Usually the larvae showing tumors undergo pupation successfully. In this case the black spots can be seen in the pupa and also in the adult. At most three or four large tumors may occur in a single individual.

Relationships to normal tissues

Microscopical examination reveals the fact that the tumor tissue lies in the body cavity of the larvae, usually between the folds of the intestine (figure 2), sometimes surrounding the heart (figure 16, plate 2). It has never been observed in a larval organ or to invade the larval tissue as does the first tumor. Displacement and crowding of organs is the only distortion noticed.

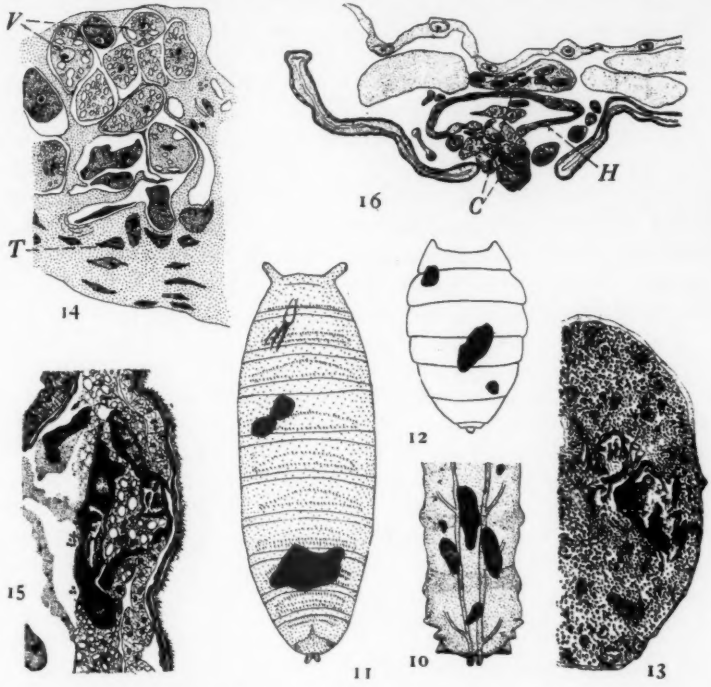
Growth and fate of tumor

The tumor tissue shows definite changes during its development. The cellular structure of the young tumor (figure 14, plate 2) is without pigment. A little later, however, pigment deposits begin, sometimes as solid knots and sometimes in great circles (see figure 2), very much as in Miss STARK's tumors. This suggests that the earliest pigmented cells have been crowded to the periphery in laminated layers by the newer cells. However, there is active growth of the tumor cells outside of the pigmented area, the cells growing in all directions and invading the spaces between the organs.

In the pupae and the adult (figures 13 and 15, plate 2) the cellular structure of the tumor seems entirely gone, leaving the pigment in place. The pigmented area before histolysis is much less than the area of tumor cells. The pigment seems to have been deposited within or between the cells, but apparently the cells containing the pigment are destroyed by histolysis, as is known to be the case with the unpigmented cells. An attempt was made to bleach some of the tumors in hydrogen peroxide and by Mayer's chlorine method. Although some of the pigment was removed its location was not apparent.

Cellular structure

The cellular structure of this tumor seems to consist of more or less scattered isolated cells embedded in a rather homogeneous non-cellular stroma, as is shown in figure 2, and in figure 14 (plate 2). The cells are spindle-shaped, rounded, or elongated, but seldom polygonal, indicating lack of crowding. In some of the tumors the cytoplasm contains many



All drawings of tumor No. 2.

FIGURE 10.—Larva showing mature tumors.

FIGURES 11 and 12.—Pupa and adult with tumors carried over from larval stage.

FIGURES 13.—Partial section of pupa showing pigmented tumor with cellular structure in advanced stage of disintegration. ×80.

FIGURE 14.—Section of part of young tumor (no pigment deposited yet). *T*, most common type of tumor cell; *V*, vesicular tumor cells embedded in non-cellular stroma. ×820.

FIGURE 15.—Portion of adult abdomen showing entire disappearance of cellular structure of tumor. ×115.

FIGURE 16.—Section showing tumor tissue (*C*) growing into cavity of larval heart (*H*). ×375.

large and small clear vesicles very much like the vesicles in the larval and adult intestinal cells. No tumor cells are found floating in the body cavity, as are found in the first tumor.

Origin

The cells of this tumor stain in much the same way as do the cell nests of the intestines (the embryonic cells destined to give rise to the adult intestine, figure 3, plate 1). The fact that some of the cells become vesicular suggests that perhaps some of them have made abortive attempts to produce the vesicles of secretion as do the functional intestinal cells. The almost universal occurrence of the tumor in the folds of the intestine also suggests that they are of intestinal cell origin.

Mortality

Of 295 non-tumor pupae isolated from the normal stock from which this tumor strain originally came, 239 hatched, i.e., 81 percent. From the tumor stock 221 tumor pupae were isolated and only 132 of them hatched, i.e., 59 percent. There is also a great mortality of tumor larvae just before pupation.

The mere mechanical occupation of space and the derangement of organs probably accounts for most of the deaths of the larvae and pupae that show tumors. Dead flies are usually observed to be those with very large tumors.

Discussion

The likelihood that either of these tumors is caused by a micro-organism is very slight, because they behave as recessive characters when hybridized with other strains (see pages 353 and 355), i.e., the tumor disappears for an entire generation and then reappears again in the next generation. Miss STARK showed that the tumor of lethal 7, is not due to a micro-organism since cultures (using all the ordinary laboratory media) from the eggs and from the tumor tissue showed no bacteria present.

HEREDITY OF THE TUMORS

Heredity of tumor number 1

This tumor occurred as a mutation in a plus bristle line that PAYNE (1918) was carrying on with selection for higher bristle number, which selection was carried on for about forty generations after the tumor appeared. He did not select for the tumor. He later determined that the extra bristles are due to at least two factors, one in the first chromosome

and one in the third. If the mutated genes which cause this tumor are linked to any of these bristle genes they would have been selected at the same time as were the factors for bristles. The tumor has remained in mass culture for four years without selection, and it would probably have disappeared if the stock had not been homozygous for the principal tumor genes. Selection for 17 generations in which pairs of flies showing tumors were used as parents did not increase the number of tumor individuals in the strain. At the same time, selection for 19 generations against tumors by pair matings of parents without tumors did not reduce the proportion of flies showing tumors. It is therefore probable that the strain was pure for the tumor genes.

To test the genetics of tumor No. 1 I crossed it out to several strains of flies with factors in well-known loci. These data are given in table 1. As many as 1235 F_1 flies were examined in these crosses, and only two developed tumors, showing the tumor to be a recessive character.

The total number of F_2 flies was 13,765, of which 64 had tumors, i.e.,

TABLE 1

CROSS	NUMBERS OF F_1 EXAMINED	F_1 WITH TUMORS	F_2 (PLUS ONE BACK-CROSS)	F_2 WITH TUMORS	PERCENT TUMORS IN F_2 CLASSES
No. 1 tumor×yellow white	181	1	5309 (F_2)	20 wild, 8 *yw*	0.8 wild, 0.3 *yw*
No. 1 tumor×b_l p_r c s_p	46	0	3186 (F_2)	7 wild	0.3 wild
No. 1 tumor×"IIIple"	186	0	3814 (F_2)	15 wild, 2 "IIIple"	0.5 wild, 0.2 "IIIple"
No. 1 tumor×eyeless	—	–	1456 (F_2)	9 wild, 3 e_y	0.8 wild, 0.8 e_y
No. 1 tumor×star dichaete (Tumor ♀×F_1 *S D* ♂)	592	0	2030 (back-cross)	43 wild, 10 *D*, 6 *S*, 12 *S D*	8.4 wild, 1.9 *D*, 1.1 *S*, 2.3 *S D*
No. 1 tumor×No. 2 tumor	230	1	836 (F_2)	38	4.5

0.5 percent. Where F_1 star dichaete males were back-crossed to the recessive tumor-strain females, there were 2030 adults, of which number 71 flies had tumors (3.6 percent).

These two percentages (0.5 and 3.6) should stand in the ratio of 1.6 to 12.5 if three non-linked recessive factors are involved. As they stand nearly in this ratio the results are not incompatible with the assumption that 3 independent recessive factors are involved. However, the inheritance is not so easily explained, as is evident when the crosses are examined separately. Hence, I am inclined to believe the similarity of ratios is merely a coincidence.

In the F_2 of the cross of tumor males to yellow white females (factors located in the left end of the first or X chromosome) 5309 adults of which 20 wild-type flies and 8 yellow white flies had tumors, or 0.8 percent and 0.3 percent, respectively. The fact that only about half as large a percentage of the yellow white flies showed tumors as did the wild-type flies, suggests that there may be a gene for tumors in the X chromosome and located near the right end. But in view of the small numbers involved in the tumor classes, it is doubtful whether any conclusion should be drawn.

The cross to the black purple curved speck strain (all factors in the second chromosome) gave 3186 F_2 adults. Of these, 7 wild-type had tumors, but none of the black purple curved speck flies had tumors, which shows that there is probably a gene for tumor in the right half of the second chromosome.

The cross to "IIIple" (roughoid hairy scarlet peach spineless sooty) gave in the F_2 3814 adults, of which 15 wild-type and 2 "IIIple" flies had tumors. Again the fact that the percentage of "IIIple" flies with tumors is only half that of the wild-type flies with tumors may be accounted for on the assumption that there is a gene for tumor in the right end of chromosome III.

The cross to eyeless (a fourth-chromosome character) gave 1456 adults of which 9 wild-type and 3 eyeless had tumors (table 1), showing that probably no gene for tumor lies in the fourth chromosome.

The differences in the percentages of tumors in the numerous F_2 classes were not greater than the difference between the percentages of occurrence of tumors in the various experiments as wholes, e.g., 0.8 for the cross to eyeless and 0.22 for the cross to black purple curved speck. That is, it is doubtful whether the above experiments have enabled us to localize the genes involved.

The strain was further tested by crossing to star dichaete (second- and third-chromosome characters, respectively, and both dominant and lethal when homozygous) and then back-crossing the F_1 star dichaete males to the tumor-strain females. Of 2030 F_2 adults 71 (3.6 percent) had tumors. The distribution of these tumor flies is as follows; 43 wild-type, 6 star, 10 dichaete, and 12 star dichaete. Since star tumor flies appeared, it follows that it is not necessary that the gene in the second chromosome be present in the homozygous condition. The results also show that the gene in the third chromosome is either not entirely recessive or not essential, because dichaete tumor flies also appeared. Star dichaete tumor flies were also present, which shows that when both the second and the third chromosomes are heterozygous in tumor genes at the same time, the

tumor may develop. Assuming the four groups of flies (wild, star, dichaete and star dichaete) to be equal in number (no record was kept, unfortunately) the percentage of wild-type flies with tumors (about 8.4) exceeds so strikingly any of the other groups (1.1, 1.9 and 2.3) that it is evident that the tumor develops most frequently in those flies in which the second and third chromosomes are homozygous for the tumor-strain chromosomes, which is the case in the wild-type flies.

The evidence, then, indicates that there are no genes in any of the chromosomes which must be homozygous, but that there are genes in each, except probably the fourth, that are essential in heterozygous form. The gene having most effect seems to be in the second chromosome. As will be shown (page 359) the environment plays in some way an important part in the production of this tumor and a more complete genetic analysis will be attempted when the environmental influences are better controlled.

Heredity of tumor number 2

This tumor line started from a single female (wild-type) which occurred in either the F_1 or the F_2 generation of a cross of lance by "IIIple" (roughoid hairy scarlet peach spineless sooty). She was heterozygous for "IIIple." This tumor-bearing female was out-crossed to wild. Of the 130 F_1 larvae none developed tumors. The non-tumor brothers and sisters from this first generation were inbred and among their offspring several tumor individuals developed which were inbred. Continued selection for tumors by pair matings (and by occasional mass cultures when pair matings failed), produced what appeared to be a pure strain of tumor flies in the tenth generation. In subsequent generations, up to the 19th, the percentages of tumors remained at about 100, although in each generation there would occur sometimes one or two individuals in which tumors could not be found. In the 19th generation, however, the percentage of tumors dropped to 66, but, without selection, jumped back to approximately 100 in the 21st generation, the 20th and 21st generations having been mass cultures. In the subsequent generations (up to the 36th, the generation of the present writing) the percentage has remained about 100 in offspring from selected pair matings. At the 26th generation I made up a mass culture of this strain and it has maintained itself to the present time with approximately 100 percent of tumor flies. The strain seems to have been homozygous since the 10th generation.

In order to test out the method of inheritance the No. 2-tumor strain was crossed to the same strains of flies used with the first tumor. These results are tabulated in table 2. The number of F_1 flies in all these crosses

that were counted and tabulated was 1180. Three of these had tumors. Therefore this tumor, like the first, is capable of developing occasionally when all its genes are heterozygous.

TABLE 2

CROSS	NUMBERS OF F_1 EXAMINED	F_1 WITH TUMORS	F_2 (PLUS ONE BACK-CROSS)	F_2 WITH TUMORS	PERCENT F_2 WITH TUMORS	PERCENT TUMORS IN F_2 CLASSES
No. 2 tumor×yellow white	103	0	1537 (F_2)	23 wild, 25 *yw*	3.1	2.9 wild, 3.2 *yw*
No. 2 tumor×*bl pr c sp*	25	0	2140 (F_2)	35 wild	1.6	2.2 wild
No. 2 tumor×"IIIple"	175	0	2650 (F_2)	24 wild	0.9	1.2 wild
No. 2 tumor×eyeless	0	0	1004 (F_2)	22 wild, 6 e_y	2.7	3.0 wild, 2.3 e_y
No. 2 tumor×star dichaete (Tumor ♀ ×F_1 *S D* ♂)	655	2	1075 (back-cross)	233	21.6	(see table 3)

The total number of F_2 flies (derived from *inter-se* crosses of F_1 flies) from crosses to strains with recessive genes was 7331 of which 145 had tumors, (1.9 percent), showing that the non-linked genetic factors involved are probably at least as numerous as three. The tumor strain was next crossed to star dichaete and the F_1 star dichaete males were back-crossed to the tumor-strain females. There were 1075 flies in F_2 of which 233 (21.6 percent) had tumors. This result also indicates multiple factors.

To test whether or not any of the factors are present in the first chromosome, males with tumors were mated to yellow white females, and the F_1 flies inbred. There were 1537 F_2 flies, of which half were yellow white as expected. There were 23 wild-type and 25 yellow white flies with tumors; eight of the yellow white tumor flies were females. This ratio gives no evidence of a factor for tumor in the first chromosome, but does not entirely exclude one if it be dominant and not closely linked to yellow. The second and third chromosomes were tested by crossing to black purple curved speck and to "IIIple," respectively. The F_1's were crossed *inter se*. The F_2's of the black purple curved speck cross gave 2140 flies of which 35 (1.6 percent) had tumors, but none of the tumor flies were black purple curved speck. This shows that there is an essential factor (or factors) in the second chromosome. The F_2 of the "IIIple" cross gave 2650 flies of which 24 (0.9 percent) had tumors, but none of the tumor flies were "IIIple", showing that there is an essential factor in the third chromosome. In the F_2 generation of the cross of tumor No. 2 to eyeless there were 1004 flies, of which 22 wild-type and 6 eyeless had tumors.

There are no factors in the fourth chromosome for tumors, since the 22 wild-type tumor flies to 6 eyeless is very near the 3 to 1 ratio that one would expect if no factors are involved. The evidence from these crosses indicates that the most essential tumor factors are located in the second and third chromosomes.

The same phenomenon appears in the heredity of both tumors, namely, that the tumor strain behaves differently when crossed to different strains. For example, there were 3.1 percent F_2 flies with tumors in the cross of tumor No. 2 to yellow white, and only 0.9 percent in the cross to roughoid hairy scarlet peach spineless sooty (table 2).

In the cross of the second-tumor strain to star dichaete there were 1075 flies in F_2 of which 233 had tumors. The data for this cross are given in table 3. One would expect equal numbers of flies of the four F_2 classes,

TABLE 3

BACK-CROSS CLASSES	TOTAL	TUMOR CLASSES	PERCENT TUMORS
Wild-type.....	268	197	73.5
Star.....	299	10	3.3
Dichaete.....	222	26	11.7
Star dichaete....	286	0	0.0
Totals.....	1075	233	21.6

namely, wild-type, star, dichaete and star dichaete. The numbers obtained, 268, 299, 222 and 286, respectively, are near enough to expectation. If the factors for tumor are located in the second and third chromosomes and are recessive, as seems apparent from the crosses to black purple curved speck and "IIIple," we would not expect to get any star, dichaete, or star dichaete flies with tumors. In the star and dichaete flies, one of the chromosomes (second or third) is heterozygous for tumor, the other homozygous; and in the star dichaete flies both the second and third are heterozygous. Since star tumor flies and dichaete tumor flies appeared, but no star dichaete tumor flies, it follows that at least one (either) of the chromosomes (second or third) must be homozygous for tumors. The preponderance of dichaete tumor flies (11.7 percent) over star (3.3 percent) indicates that the factors in the second chromosome are more influential in tumor development than those of the third. Also, the large preponderance of wild-type flies with tumors (73.5 percent) as compared with the other groups (3.3 percent, 11.7 percent, and 0.0 percent) shows that the

factors in the second and third chromosomes are very much more influential when both are homozygous. However, since all stock flies have tumors and only 73.5 percent of the wild-type flies in F_2 of this back-cross have them, it follows that some of the factors for tumor are located in the first or fourth chromosome or both, for, as noted above, both classes of flies have the same type of second and third chromosomes.

Summing up the results of these crosses, it is evident that three factors, at least, located in three different chromosomes, are involved in the production of the tumor, and that in most cases some of them have to be present in homozygous condition. The first or fourth chromosome, probably both, was shown to contain recessive factors that modify (increase) the rate with which flies develop tumors, but neither chromosome is essential to tumor development when considered alone. The second and third chromosome each contain an essential factor, but each is capable of causing tumor development in the heterozygous condition if the other is present in the homozygous condition.

Cross of tumor number 1 and tumor number 2

The two strains of tumor flies were crossed. Only one of the F_1 flies developed tumors, but the tumor reappeared again in the F_2 generation in considerable numbers (4.6 percent). These facts show conclusively that some of the essential factors for the development of the two tumors are entirely different, otherwise we should have got all of the F_1 flies with tumors. The percentage of F_2 flies obtained (4.6 percent) is considerably more than the sum (2.61) of the percentages of the F_2 flies of each tumor (0.46 and 2.15) when crossed to other strains. This suggests that some of the genes of one may modify or interact with some of the genes of the other, thus increasing the number of tumor flies in F_2 over what would be expected if the genes of the two are entirely different.

General statement

The genetic analysis of the tumors given above has not exhausted all the possibilities, and the statements made are meant only as an indication of the many factors involved. It is hoped that by more carefully planned experiments a complete and satisfactory analysis of both tumors may be made.

THE INFLUENCE OF THE ENVIRONMENT

It was early noticed in tumor No. 1 that sometimes there were many more flies with tumors than at other times. Experiments were made to discover the cause of this difference. It was found that the number of

individuals developing tumors depends in some way upon the number of larvae living upon a definite amount of food. An ordinary glass slide was covered with a known amount of food (a thin layer of untreated banana) and then a known number of newly hatched larvae were placed upon it and allowed to develop. The number developing tumors was noted. In those bottles containing from 1 to 20 larvae 32 percent developed tumors

TABLE 4

NUMBER OF LARVAE PER BOTTLE	NUMBER OF BOTTLES	TOTAL LARVAE	AVERAGE PER BOTTLE	NUMBER WITH TUMORS	PERCENT WITH TUMORS	TOTAL ADULTS WITH TUMORS	PERCENT ADULTS FROM TUMORS
1 to 20	9	135	15	44	32	5	11
20 to 50	11	351	32	82	23	36	44
50 to 100	8	556	69	115	20	49	43
Over 100	10	1418	142	216	15	73	34

(table 4); in those bottles with 20 to 50 larvae there were 23 percent of tumor individuals; in those with 50 to 100, 20 percent; and in those with over 100, 15 percent. This declining series of percents, which is inversely proportional to the number of larvae placed upon the unit of food, is similar to the result obtained in an earlier experiment in which the percent of tumor individuals varied inversely with the number of parent flies used, whether single, double or triple pair matings, etc.

Furthermore, the larvae from the bottles with less than 20 larvae in them had the tumors much larger and had many more additional small tumors than those larvae from bottles containing more than 20 larvae (table 4). This is further shown by the fact that in the bottles which have from 1 to 20 larvae there is a mortality from tumors, of 89 percent, while in those bottles with more than 20 larvae there is a mortality of only 56 percent to 65 percent.

In the bottles with few larvae the food becomes very much contaminated with bacteria and large fungus growths, the banana furnishing an excellent medium for their growth; the food becomes very foul. In the bottles with many larvae, however, the growth of both the bacteria and the larger fungi is kept down and the food has the appearance of being clean. It seems possible that the enormously different environmental conditions presented here are responsible for the difference in the number of larvae which develop tumors. Again, in bottles with few larvae the larvae have an abundance of food, while in the bottles with many larvae the food is

scarce, relatively, and the larvae pupate frequently when only partly grown. This would suggest that starvation is inimical to tumor development, a suggestion made before by workers with tumors. We hope to follow out this relation more thoroughly and report upon it in a future paper.

The second tumor seems to kill more of the flies in the larval and pupal stages at some times than at others, the tumors apparently being larger and greater in number in each larva, which suggests that perhaps the environment may play some part in the development of this tumor.

DISCUSSION

It is apparent that these tumors are similar in many respects to those described by Miss STARK. In all probability all of them develop from larval tissue and most commonly from the embryonic rudiments of the larva, i.e., from the cells destined to give rise to the structures of the adult flies. All deposit black pigment, and all are hereditary.

These four tumors together with another one which I have, and another in the possession of Doctor F. PAYNE, make it quite probable that tumors in the fruit fly are of common occurrence. Miss STARK's two tumors and my two here reported are decidely different from each other in their time of development, final destiny, malignancy, and mode of inheritance, making it certain that these tumors are due in part at least to different and independent mutations in different genes. The lethal 7 tumor proved to be due to a single unit factor that was sex-linked, but Miss STARK'S benign tumor and my two tumors are due to several factors (multiple factors) and the heredity is very complex, but probably not more so than ALTENBURG and MULLER'S (1920) truncate wing character.

That the development of the first tumor here described depends in some way upon environmental conditions is not unique, since other hereditary characters are known which depend upon a proper environment for their normal appearance, e.g., Miss HOGE'S (1915) flies with supernumerary legs which show the extra legs only at low temperatures.

A new and interesting feature is exhibited by the two tumors here reported in that the cellular structure is entirely lost during pupation. It is apparently not due to phagocytosis, since I can find but very few phagocytes at the edges of the tumors or within during the histolysis of the normal tissue, and besides, phagocytosis would probably remove pigment as well as cells. The tumor's disintegration must be due, therefore, to something characteristic of its constitution or to some chemical substance produced by the early pupa itself. If these tumors are derived

from the imaginal discs, as the evidence seems to show, it is not clear why they disintegrate, since the normal imaginal discs are immune to histolysis. Possibly the substance causing their destruction is very specific for this particular tumor tissue.

SUMMARY

1. Two new tumors, in totally unrelated strains of flies, are here recorded.

2. They both deposit black pigment in later stages.

3. The tumors are probably derived from embryonic rudiments (imaginal discs) (in the first tumor the neuroblast and hypodermal tissue) of the larva. In the first tumor many small tumors are often present that may be metastases arising from cells broken loose from the primary tumor.

4. The tumors differ in their cell structure, location in the larva, number, and malignancy.

5. It is unlikely that the tumors are caused by a micro-organism.

6. Both tumors are hereditary and depend upon multiple factors for their development. Some of the factors can be classed as essential and others as modifying factors, but the dominance and recessiveness of these factors are not clear-cut and their interrelationships are very complicated. It was found that some of the factors, at least, cause a greater number flies to have tumors when homozygous for those factors than when heterozygous.

7. Both tumors behave as recessives when crossed to other strains and also when crossed to each other, showing that most of their genes are not the same, although there was evidence that they had a factor or factors in common.

8. Both tumors show different percentages of tumor flies in the F_2 when crossed to different strains, showing that the action of the tumor factors is modified in some way by genes peculiar to the different strains to which they were crossed.

9. The first tumor is dependent for its full realization upon certain environmental conditions not well understood at present. The second tumor also, but in a lesser degree, is probably in some way dependent upon the environment for its fullest development.

LITERATURE CITED

ALTENBURG, EDGAR, and MULLER, HERMAN J., 1920 The genetic basis of truncate wing,—an inconstant and modifiable character in Drosophila. Genetics **5**: 1–59.

BRIDGES, CALVIN B., 1916 Non-disjunction as proof of the chromosome theory of heredity. Genetics **1**: 1–52, 107–163.

BRIDGES, CALVIN, B., and MORGAN, THOMAS H., 1923 The third-chromosome group of mutant characters of *Drosophila melanogaster*. Carnegie Inst. Washington Publ. 327, 251 pp.

HOGE, MILDRED A., 1915 The influence of temperature on the development of a Mendelian character. Jour. Exp. Zoöl. **18**: 241–297.

PAYNE, F., 1918 An experiment to test the nature of the variations on which selection acts. Indiana University Studies 36, 45 pp.

STARK, MARY B., 1918 An hereditary tumor in the fruit fly, Drosophila. Jour. Cancer Res. **3**: 279–302.

1919 a An hereditary tumor. Jour. Exp. Zoöl. **27**: 507–529.

1919 b A benign tumor that is hereditary in Drosophila. Proc. Nation. Acad. Sci.

SEASONAL DIFFERENCES IN SEX RATIO, LITTER SIZE AND BIRTH WEIGHT OF THE ALBINO RAT UNDER UNIFORM LABORATORY CONDITIONS

FRANK BLAIR HANSON AND FAITH NICKELL SHOLES
Department of Zoölogy, Washington University, Saint Louis, Missouri

Received April 5, 1924

TABLE OF CONTENTS

	PAGE
INTRODUCTION	363
Sex ratio	364
Litter size	366
Birth weight	366
CONCLUSION	367
LITERATURE CITED	367

INTRODUCTION

The immediate purpose of this study was a practical one. For the past three years the senior author has been treating successive generations of albino rats with alcohol fumes (HANSON and HANDY 1923). Both treated and control animals in all generations came from a single pair of rats. During the course of the experiment, however, the control animals have gained an entire generation over the treated. That is, at the date of writing there are eight generations of controls, but only seven of the alcoholic.

It is necessary to compare control and treated generation by generation. Since these two groups are now being produced at different seasons of the year, due to the greater rapidity with which the controls breed, we sought to ascertain just what the seasonal differences in sex ratio, litter size and birth weight are in laboratory animals kept under approximately uniform conditions throughout the year. Any difference exhibited between rats born in the winter and those at any other season could then be used as a correction factor in the comparison of treated and alcoholic rats of any generation.

Our material for this study consists of all the control animals produced during the first five generations. These animals were divided into four groups; all those born in June, July and August make one group, those of September, October and November another, while December, January and February and March, April and May constitute the winter and spring groups, respectively. For a comparison of sex ratio, litter size and birth

weight these are the four best divisions of the year possible, but in the study of the growth curve it is not so good, for those animals born near the end of one season have almost their entire growth period falling in the succeeding season. However, this is not too serious when it is considered that any division of the year is an arbitrary one and would have the same objection as the one adopted. What is wanted is a correction factor which may be applied to the alcoholic experiment and this seems to be supplied by this method.

The five generations of control animals used in this study were produced over a period of two years; hence, each group includes litters born during two successive summers, two successive winters, etc.

The animals are kept in large comfortable cages, with a permanent water supply, and are fed with cooked food after a WISTAR INSTITUTE recipe. To this are added lettuce and dog biscuit. The diet seems to be a highly successful one judged by the condition of the animals and their breeding performance. The colony is housed in a basement room the floor of which is approximately level with the ground. Good light and ample ventilation are provided. The heating system for this room is separate from that of the rest of the building and can be controlled within reasonably narrow limits both day and night. With two stories of the building above the animal room the summer temperature is never excessive. It is probably safe to state that the fluctuations in temperature throughout the year are from 15° to 27°C, but the lower and higher temperatures indicated are not frequently met with. For mammals this does not seem to be a harmful variation.

SEX RATIO

Table 1 shows the variation constants for sex ratio, litter size and birth weight during the four seasons of the year, together with the number of rats upon which each constant is based. It will be seen that the number of animals is large in each case and the probable errors are correspondingly small.

The lowest sex ratio is found in the spring months and the highest during the winter months. However, when these two extreme sex ratios are compared, regard being taken of their probable errors, it is evident that the difference between them is not a significant one:

$$49.41 \pm 3.83 - 43.93 \pm 3.77 = 5.48 \pm 5.37$$

The low spring sex ratio may also be compared with that of the summer months and the fall months. With the summer months we have the smallest difference of all and our constants appears as follows:

$$46.00 \pm 4.17 - 43.93 \pm 3.77 = 2.07 \pm 5.62$$

The same method of comparison between spring and fall sex ratios yields the following results:

$$49.31 \pm 4.17 - 43.93 \pm 3.77 = 5.38 \pm 5.62$$

TABLE 1

A table showing seasonal differences in sex ratio, size of litter and birth weight of albino rat.

MONTHS	SEX RATIO	LITTER SIZE	BIRTH WEIGHT	NUMBER OF RATS
June July August	46.00±4.17	6.06±0.27	5.51±0.14	200
March April May	43.93±3.77	5.97±0.31	5.63±0.09	171
December January February	49.41±3.83	6.54±0.27	5.54±0.06	157
September October November	49.31±4.17	6.26±0.41	5.43±0.05	136

There is one direct observation in the literature, on seasonal differences in the sex ratio of the albino rat. DONALDSON (1915), quoting KING and STOTSENBURG (1915), says, that

"in a thriving colony a ratio of about 108 males is to be expected. This, however, is subject to a seasonal variation. At the two periods of reproductive activity—in the spring (March-May) and again in the autumn (September-November) the proportion of males (the sex ratio) is low."

Our data agree with the above statement as far as the sex ratio of the spring months is concerned. It is the lowest of the four seasons of the year. But the sex ratio in the autumn months is almost as high as that of the winter, the highest of all. It should also be pointed out that while the spring sex ratio is *actually* lower than that of the other three seasons, it is not *significantly* lower, as indicated by the difference divided by the probable error discussed above.

It is evident that in comparing sex ratios of litters born at different seasons of the year under the conditions prevailing in this laboratory no correction factor is necessary; for the difference between the most extreme groups is not statistically significant, due regard being paid to the probable error.

LITTER SIZE

KING and STOTSENBURG (1915) found that litter size did not appear to be influenced by the season. Table 1 shows that this is true also for our colony. Taking again, as for sex ratio, the two most extreme constants, those for the spring and winter months, it is evident that this slight difference has no meaning:

$$6.54 \pm 0.27 - 5.97 \pm 0.31 = 0.57 \pm 0.41$$

Comparing the spring litter size with that of the summer and autumn (constants from table 1) the following two lines tell the same story of differences that are negligible:

$$6.06 \pm 0.27 - 5.97 \pm 0.31 = 0.09 \pm 0.41; \text{ and,}$$
$$6.26 \pm 0.41 - 5.97 \pm 0.31 = 0.29 \pm 0.51$$

In two of the three cases the probable error is larger than the difference, and once it approaches closely the difference. Accordingly, the conclusion seems valid that under the conditions prevailing in this colony, litter size is not affected by the seasons, and comparisons between litters born at different periods of the year are justified.

BIRTH WEIGHT

Birth weight is remarkably constant in these animals, whether they happen to be born in summer or winter, spring or autumn. It is less variable than sex ratio or litter size, the total range from the lowest to the highest is only 0.20 grams.

The birth weight is highest in the spring months (see table 1) and lowest in the autumn. As the litter size is lowest in March, April and May, there appears to be some degree of correlation between birth weight and litter size; i.e., as the litter size goes down, the birth weight increases, and *vice versa*.

In the comparison of the two extreme constants, those for spring and autumn, we get the following statement:

$$5.63 \pm 0.09 - 5.43 \pm 0.05 = 0.20 \pm 0.10$$

Since the difference is only twice its probable error, it does not appear that there is a statistical difference between the birth weights of the spring and fall litters.

The other two comparisons that might be made are those between summer and autumn, and autumn and winter. These give the following results:

$$5.51 \pm 0.14 - 5.43 \pm 0.05 = 0.08 \pm 0.15; \text{ and,}$$
$$5.54 \pm 0.06 - 5.43 \pm 0.05 = 0.11 \pm 0.08$$

From the above data it cannot be held that the birth weight of our albino rats is changed in a significant manner by reason of accident of birth during any particular season of the year.

CONCLUSION

In a paper to follow this one data will be given for sex ratio, litter size and birth weight of ten generations of control and alcoholic rats. Some of the groups there compared were born at different seasons of the year. That such comparisons are valid seems to be borne out by the data presented in this paper.

LITERATURE CITED

DONALDSON, H. H., 1915 The rat. v+278 pp. Philadelphia: The Wistar Institute.

HANSON, F. B., and HANDY, V., 1923 The effects of alcohol fumes on the albino rat. I. Introduction and sterility data for the first treated generation. Amer. Nat. **57**: 532–544.

KING, H. D., and STOTSENBURG, J. M., 1915 On the normal sex ratio and the size of the litter in the albino rat (*Mus norvegicus albinus*). Anat. Rec. **9**: 403–420.

CORRELATIONS OF BODY WEIGHT, BODY LENGTH, AND TAIL LENGTH IN NORMAL AND ALCOHOLIC ALBINO RATS

FRANK BLAIR HANSON AND FLORENCE HEYS
Washington University, Saint Louis, Missouri

Received April 5, 1924

The material upon which the correlations in this paper are based consists of three generations of white rats. Part of these were treated daily with alcoholic fumes in an air-tight tank; the remainder were controls. But both treated and controls were descendants of a single pair of rats, which was derived from an inbred race of WISTAR INSTITUTE rats. All matings during the experiment were sister by brother within the litter. It is apparent that the material was fairly on the way to a condition of homozygosity.

This paper is one of a series on the effects of alcohol fumes on the albino rat. For a detailed account of the general aspects of the experiment see the introductory paper by HANSON and HANDY (1923).

The rats were weighed at birth, and thereafter every ten days, except in certain groups which were weighed at twenty-day intervals. All weighings throughout the three generations were made and recorded by the senior author. Taking the body length and tail length in the living animal presented some difficulty, but the following method was devised and used in all measurements. All measuring was done by the senior author with the aid of the same assistant throughout. The rat to be measured was placed with its back in the palms of the two hands, the thumb and forefinger of the left hand grasped the tail firmly near its base, the thumb and forefinger of the right hand held the two ears and part of the loose skin of the neck. As the rats were handled every day and very gentle they did not object to this method of handling. By the use of calipers the millimeter distance from the tip of the nose to the anus, and from the anus to the tip of the tail was obtained. The tail lengths were undoubtedly obtained with very considerable accuracy, the body length being a more difficult matter. However, with practice, approximately the same amount of stretching of the body for measurement was obtained in all cases. As one person held the rats throughout the experiment, the personal equation was reduced to a minimum, and it is thought that such inaccuracies as are inherent in the method were reduced to a minimum and will practically disappear or balance in the averages.

The completed raw data were turned over to the junior author, who made all the correlations and constructed the plate of graphs. The correlations were made with the aid of the Otis Correlation Charts, devised by ARTHUR J. OTIS. By this method the amount of labor involved in such a large number of computations was materially reduced.

THE DATA

Two questions are apparently answered by the data. These are, (1) Is there correlation, and how much, between body weight and tail length, and between body length and tail length in the albino rat? (2)

TABLE 1

Table showing correlation coefficients and probable errors.

AGE IN DAYS	BODY WEIGHT ON BODY LENGTH		BODY WEIGHT ON TAIL LENGTH		BODY LENGTH ON TAIL LENGTH	
	♂ ♂	♂ ♂	♂ ♂	♂ ♂	♂ ♂	♂ ♂
	Control	Treated	Control	Treated	Control	Treated
20	.8086±.0286	.8842±.0201	.9678±.0212	.8394±.0287	.8790±.0258	.8958±.0227
30	.8386±.0230	.8439±.0284				
40	.8814±.0196	.8712±.0255	.8954±.0172	.9595±.0219	.8018±.0245	.7602±.0315
50	.8022±.0287	.7108±.0217				
60	.7608±.0342	.8053±.0420	.9628±.0190	.9536±.0196	.9025±.0239	.8762±.0251
70	.8787±.0231	.8094±.0486				
80	.8145±.0218	.8791±.0310	.9464±.0197	.8903±.0333	.8990±.0168	.8562±.0308
	♀ ♀	♀ ♀	♀ ♀	♀ ♀	♀ ♀	♀ ♀
20	.7094±.0327	.7600±.0348	.9184±.0129	.9400±.0650	.8153±.0215	.8837±.0247
30	.8816±.0190	.8505±.0035				
40	.8452±.0270	.8014±.0340	.8413±.0264	.8017±.0434	.7805±.0278	.8402±.0288
50	.8792±.0192	.8684±.0308				
60	.8857±.0230	.7574±.0494	.8560±.0245	.8527±.0312	.8991±.0215	.8862±.0268
70	.8349±.0266	.8271±.0491				
80	.7921±.0266	.8849±.0347	.8567±.0382	.9725±.0375	.8963±.0192	.8530±.0446

Does the administration of alcoholic fumes alter these correlations in any given manner?

The first question is answered by reference to the summary of correlations in table 1. Of the sixty correlation constants there listed, eight are above 0.70; forty-three are above 0.80; and nine are above 0.90.

The lowest correlation in the entire series, (0.7094 ± 0.0327) is that of twenty-day females, body weight on body length. The highest rise to 0.9725 ± 0.0375 in eighty-day females, body weight on tail length, an extremely high correlation, 1.0000 being complete correlation.

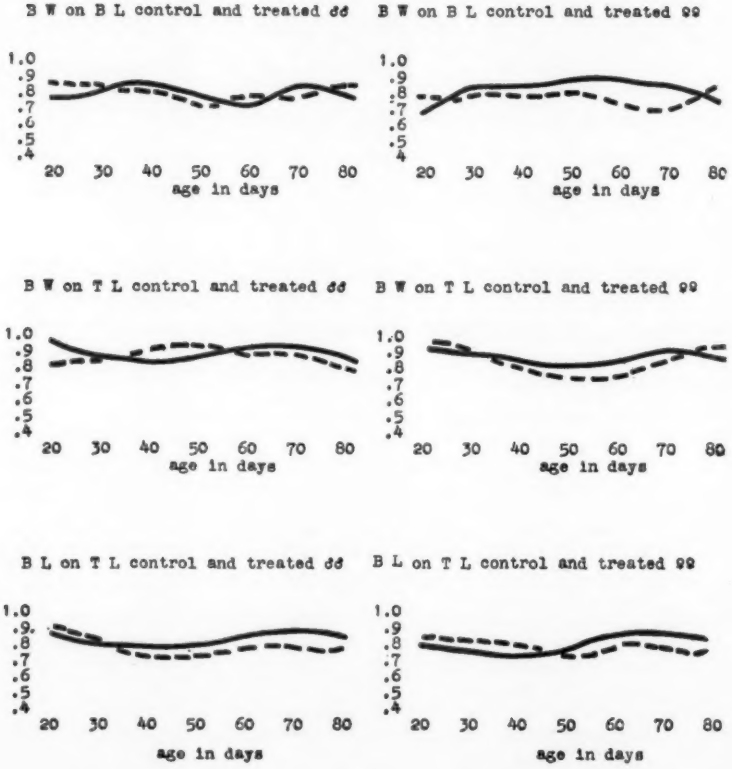

B W on B L control and treated ♂♂
B W on B L control and treated ♀♀
B W on T L control and treated ♂♂
B W on T L control and treated ♀♀
B L on T L control and treated ♂♂
B L on T L control and treated ♀♀
1.0
.9
.8
.7
.6
.5
.4
20 30 40 50 60 70 80
age in days

FIGURE 1.—Graphs showing correlations plotted against age in days, based on the computations in table 1. The solid line is that of the controls; the broken line, the treated. BW = body weight; BL = body length; TL = tail length.

An inspection of table 1 indicates that the correlation constant neither increases nor decreases with age. The distribution of the higher and lower constants seems to be purely a random one.

Nor can any significant difference be detected in the constants of the two sexes. The female correlation constants contain both the lowest (0.7094 ± 0.0327) and the highest constant (0.9725 ± 0.0375), but the greater range of correlation constants in the females is, however, an insignificant one. It may be stated that in these data the females are actually more variable than the males, but to a degree that falls short of having any meaning.

The effects of the alcoholic treatment, while markedly lowering the mean body weight in this material, did not disturb the relations of body weight to body length and tail length. Figure 1 shows graphically the correlations of tests and controls over the different age periods for which the computations were made. It is apparent that the treatment had not significantly changed the character of the graphs in alcoholic animals.

One is impressed by the uniformity of the constants, whatever way the comparison is made.

LITERATURE CITED

HANSON, F. B., and HANDY, V., 1923 The effects of alcohol fumes on the albino rat. I. Introduction and sterility data for the first treated generation. Amer. Nat. **57**: 532–544.

A GENETIC ANALYSIS OF VARIEGATION[1]*

WILLIAM H. EYSTER
University of Missouri, Columbia, Missouri

Received April 10, 1924

TABLE OF CONTENTS

	PAGE
INTRODUCTION	372
Previous studies of variegation	373
Terminology	376
Source of material	376
Methods of study	376
Acknowledgements	378
Inconstancy of orange pericarp	378
Variation in color intensity of orange pericarp	378
Color changes in orange pericarp	379
Relation of color intensity to rate and direction of color changes	382
Frequency of changes from orange to red	382
Frequency of changes from orange to lighter orange, light variegations or colorless	383
Frequency of changes from orange to variegation	383
Types of variegation originating from orange pericarp	384
Inheritance of the different variegation patterns	385
Inheritance of the light-variegation patterns	385
Inheritance of the heavy-variegation patterns	387
Relation between color intensity of orange pericarp and type of variegation	388
Color changes in the variegated patterns	388
Frequency of changes in light variegations	388
Frequency of changes in heavy variegations	389
Frequency of changes to dark crown in the variegated types	390
Frequency of changes from heavy to light variegations	391
Reverse changes from variegation to orange pericarp	391
Changes from pink variegation to red variegation	391
Color changes in the cherry-red pericarp that originated from orange pericarp	392
Reverse changes from cherry-red to orange	392
Reverse changes from cherry-red to variegation	392
Stability of colorless pericarp originating from orange pericarp	392
Summary of color changes in the orange-pericarp series	393
Interpretation and discussion	993
The nature of the gene	394
The nature of variegation	397
SUMMARY	398
LITERATURE CITED	398
APPENDIX—Tables 1 to 10	400

INTRODUCTION

Variegations refer to the presence of contrasting characters in the somatic tissues of individual plants and animals. They have been found

* The GALTON AND MENDEL MEMORIAL FUND has contributed toward the colored plate accompanying this article.

[1] This study was made in the Department of Field Crops, MISSOURI AGRICULTURAL EXPERIMENT STATION.

in the higher animals, affecting coat color, and in many species of plants, affecting flower color, seed-coat color, the distribution of the chloroplastid pigments, and, less frequently, morphological characters, as the decompounding of leaf parts. Every variegation has two or more different types around and between which it varies, but to which it is limited. Variegated pericarp of maize fluctuates between colorless and red with all possible patterns, ranging from fine to coarse, and from light to heavy. Leaves variegated for the chloroplastid pigments fluctuate between green and white, or green and yellow, with a large number of intergrading patterns. In several species of ferns compounding of the fronds occurs in patches of all sizes. The variety consists of individuals with compound fronds, individuals with typical fronds, and individuals with all possible combinations of typical and compounded pinnae.

Wherever they occur, variegations are fundamentally alike in their inconstancy, giving rise more or less frequently to individuals with only one of the contrasting characters of the variegation, with a series of intergrading individuals extending from one extreme to the other. The more extreme variations, as for example a red kernel on a variegated ear of maize or a branch with colorless leaves on a variegated plant, have been variously called sports, bud-sports, mutations, somatic mutations, and the like. Since the same kind of variability is common to all variegations, in plants as well as in animals, there must be some fundamental cause for it. The present paper is concerned with a genetic interpretation of the inconstancy peculiar to variegated plants and animals.

Variegations in pericarp color of maize furnish excellent material for a genetic analysis because of the many different variegation patterns, the high frequencies of color and pattern changes, and the ease with which an abundance of suitable material may be obtained.

PREVIOUS STUDIES OF VARIEGATION

De Vries (1905) was among the first to call attention to the inconstancy so common in variegated plants. He studied a variety of Antirrhinum with variegated flower color, in which he found a continuous range of variation from narrow- to broad-striped forms. These types, the narrow-striped less frequently than the broad-striped, gave rise to occasional self-red individuals. De Vries (1910) also studied a variety of *Hesperis matronalis* L. which varied in flower color from whitish to violet and gave rise more or less frequently to variegations. The occurrence and behavior of these flower-color variations seem to be exactly

A GENETIC ANALYSIS OF VARIEGATION[1]*

WILLIAM H. EYSTER
University of Missouri, Columbia, Missouri

Received April 10, 1924

TABLE OF CONTENTS

	PAGE
INTRODUCTION	372
Previous studies of variegation	373
Terminology	376
Source of material	376
Methods of study	376
Acknowledgements	378
Inconstancy of orange pericarp	378
Variation in color intensity of orange pericarp	378
Color changes in orange pericarp	379
Relation of color intensity to rate and direction of color changes	382
Frequency of changes from orange to red	382
Frequency of changes from orange to lighter orange, light variegations or colorless	383
Frequency of changes from orange to variegation	383
Types of variegation originating from orange pericarp	384
Inheritance of the different variegation patterns	385
Inheritance of the light-variegation patterns	385
Inheritance of the heavy-variegation patterns	387
Relation between color intensity of orange pericarp and type of variegation	388
Color changes in the variegated patterns	388
Frequency of changes in light variegations	388
Frequency of changes in heavy variegations	389
Frequency of changes to dark crown in the variegated types	390
Frequency of changes from heavy to light variegations	391
Reverse changes from variegation to orange pericarp	391
Changes from pink variegation to red variegation	391
Color changes in the cherry-red pericarp that originated from orange pericarp	392
Reverse changes from cherry-red to orange	392
Reverse changes from cherry-red to variegation	392
Stability of colorless pericarp originating from orange pericarp	392
Summary of color changes in the orange-pericarp series	393
Interpretation and discussion	993
The nature of the gene	394
The nature of variegation	397
SUMMARY	398
LITERATURE CITED	398
APPENDIX—Tables 1 to 10	400

INTRODUCTION

Variegations refer to the presence of contrasting characters in the somatic tissues of individual plants and animals. They have been found

* The GALTON AND MENDEL MEMORIAL FUND has contributed toward the colored plate accompanying this article.

[1] This study was made in the Department of Field Crops, MISSOURI AGRICULTURAL EXPERIMENT STATION.

in the higher animals, affecting coat color, and in many species of plants, affecting flower color, seed-coat color, the distribution of the chloroplastid pigments, and, less frequently, morphological characters, as the decompounding of leaf parts. Every variegation has two or more different types around and between which it varies, but to which it is limited. Variegated pericarp of maize fluctuates between colorless and red with all possible patterns, ranging from fine to coarse, and from light to heavy. Leaves variegated for the chloroplastid pigments fluctuate between green and white, or green and yellow, with a large number of intergrading patterns. In several species of ferns compounding of the fronds occurs in patches of all sizes. The variety consists of individuals with compound fronds, individuals with typical fronds, and individuals with all possible combinations of typical and compounded pinnae.

Wherever they occur, variegations are fundamentally alike in their inconstancy, giving rise more or less frequently to individuals with only one of the contrasting characters of the variegation, with a series of intergrading individuals extending from one extreme to the other. The more extreme variations, as for example a red kernel on a variegated ear of maize or a branch with colorless leaves on a variegated plant, have been variously called sports, bud-sports, mutations, somatic mutations, and the like. Since the same kind of variability is common to all variegations, in plants as well as in animals, there must be some fundamental cause for it. The present paper is concerned with a genetic interpretation of the inconstancy peculiar to variegated plants and animals.

Variegations in pericarp color of maize furnish excellent material for a genetic analysis because of the many different variegation patterns, the high frequencies of color and pattern changes, and the ease with which an abundance of suitable material may be obtained.

PREVIOUS STUDIES OF VARIEGATION

DE VRIES (1905) was among the first to call attention to the inconstancy so common in variegated plants. He studied a variety of Antirrhinum with variegated flower color, in which he found a continuous range of variation from narrow- to broad-striped forms. These types, the narrow-striped less frequently than the broad-striped, gave rise to occasional self-red individuals. DE VRIES (1910) also studied a variety of *Hesperis matronalis* L. which varied in flower color from whitish to violet and gave rise more or less frequently to variegations. The occurrence and behavior of these flower-color variations seem to be exactly

like the color and pattern variations in orange pericarp as described in this paper. Of the color variations in Hesperis DE VRIES says:

"A glance at a large bed reveals the general distribution of color. At once the pale flowers are seen to be in the majority, whereas the whitish on the one hand and the lilac on the other are obviously rarer. The violet stand out conspicuously because they are not connected with the rest by any gradations. Except for this the variation is so continuous that it is almost impossible to express it in numbers. I have tried to arrange the plants in groups and to count the numbers of each group. And I give the numbers obtained in this way, only with the object of conveying to the reader the general impression which a bed makes on the observer, for it is inevitable that the limits between the groups should be somewhat arbitrary. . . ."

"For the purposes of these color valuations I picked a flowering cluster, if possible the terminal one, from each of the plants on a bed, brought them to my house and sorted them there. I made the following more or less clearly defined groups:

W. Whitish, always without stripes.
  W_1. Almost white; buds and withering petals almost white.
  W_2. White suffused with lilac; not darker when withering.
  W_3. Very pale lilac; buds lilac; only slightly darker when withering.
L. Lilac, sometimes striped or spotted.
  L_1. Definitely lilac, although pale; darker than W_3.
  L_2. Lilac; half as dark as V.
V. Violet, the color of the typical species."

The observations of DE VRIES indicate that the seed from the whitish-flowered plants give rise to progenies that are prevailingly whitish-flowered, but a few plants with lilac flowers and still fewer plants with violet flowers are produced. The seeds of the lilac-flowered plants produced plants with whitish flowers, plants with lilac flowers, and plants with violet flowers in approximately equal numbers. The lilac-flowered plants also produced a number of distinct variegations, which, DE VRIES says, can be split by selection in a plus and in a minus direction, into a pale lilac, or finely striped race on the one hand, and on the other into the dark lilac and frequently striped dame's violet.

CORRENS (1910) reported a study of the inheritance of the self-green condition which appeared as a bud sport on variegated-leaved plants of *Mirabilis Jalapa*, and of the self-red color of the flower which appeared in striped-flowered plants of the same species.

HARTLEY (1902) gave an account of an experiment with variegated maize. In a colorless or light-variegated strain he found an ear with a relatively large patch of red self-colored kernels. These red kernels were found to produce both red and white ears and were doubtless due to a somatic change from variegation, as suggested by EMERSON (1913).

EAST and HAYES (1911) found in a field of dent maize of unknown parentage a partly variegated and partly red ear, similar to the one described by HARTLEY. The red kernels were found to be heterozygous for self-color and no doubt represented a somatic color change from a light variegation.

EMERSON (1913, 1917) was the first to make a careful study of variegation in maize. He attributed the changes in pericarp color or variegation pattern to mutations in the pericarp gene in the course of development, and found that the changes from variegation to self-color are reversible but with different frequencies. The amount of tissue affected by any change in a gene varies from a fractional part of a single kernel to a patch covering a number of kernels and sometimes an entire ear, depending upon the stage in development at which the change occurred. He also found that in homozygous variegated and self-colored strains only one of the homologous genes mutates at a given time; and in plants heterozygous for colorless pericarp it is the variegation or self-color gene, never the colorless one, that changes. EMERSON has clearly shown that his interpretation of the observed changes in maize pericarp fits equally well the results found by DE VRIES in Antirrhinum, and the changes in leaf color and flower color of Mirabilis reported by CORRENS.

HAYES (1917) found mosaic pericarp, a very coarsely variegated type, to be equally or even more variable than the variegated patterns studied by EMERSON. He states that his results could be brought into line with EMERSON'S by assuming a separate factor for medium mosaic, M_m, which is allelomorphic to a factor for light mosaic, M_l and by further assumption of frequent germinal variations of M_l to M_m and *vice versa*. HAYES regards the hypothesis of slight germinal variations for the mosaic factors to be a simpler explanation of the results obtained. These germinal variations are thought to be due to certain heterozygous combinations which produce germinal instability.

GREGORY (1911) described some experiments with a variegated, or flaked, type of Primula, but made no mention of the occurrence of self-colored flowers on the variegated plants. His data indicate that the flaked type is inherited as a simple Mendelian recessive character.

PUNNETT (1922) described an interesting type of variegation in Lathyrus. The flowers of the strain in which the variegated individuals occur normally have a deep red standard and purple wings. In the variegated flowers the purple is replaced in part by a purplish pink, and thus forms a typical variegation. PUNNETT assumes that the variegated plants produce three kinds of gametes: (1) a purple gamete which is pure, (2) a red

gamete which also is pure, and (3) a flaked gamete which is a mosaic and thus impure. In inheritance the variegated plants are inconstant.

TERMINOLOGY

In maize there is a large series of multiple allelomorphs concerned in the production of pigments in the tissues of the cob and pericarp. An extended study of many of these allelomorphic genes was made by Doctor E. G. ANDERSON (unpublished). It was earlier believed by EMERSON (1911) that color in the pericarp and color in the cob are due to separate but closely or completely linked genes. More recent investigations, especially EMERSON'S (1913, 1917) studies of variegated pericarp and ANDERSON'S study of allelomorphism, indicate that a single gene controls the development of color in both pericarp and cob. The genes for cob and pericarp color mentioned in this paper are listed below:

RR, pericarp red, cob red.
WR, pericarp white (colorless), cob red.
WW, pericarp white (colorless), cob white.
OW, pericarp orange, cob white.
V_1V_1, V_2V_2, . . . V_nV_n, variegated pericarp, variegated cob.
RW, pericarp red, cob white.

The genes for colorless pericarp and white cob, colorless pericarp and red cob, and some of the genes for red pericarp and red cob are highly stable, while those for orange pericarp and white cob, and variegated pericarp and variegated cob are quite unstable. It has not yet been definitely determined whether the gene for orange pericarp and white cob and the genes for variegated pericarp and cob to which it gives rise belong to the allelomorphs of *P* described by ANDERSON. Use was made of the stable genes in the study of the unstable orange-pericarp and variegated-pericarp genes, as will be indicated later.

SOURCE OF MATERIAL

All of the material used in the present study originated in pedigreed cultures from a single ear, an illustration of which is shown in figure *A*, plate 1. It has a dilute red or orange pericarp and a white cob. Orange pericarp was found to be inconstant, giving rise to colors ranging from whitish to deep cherry red, and also a number of distinct variegations originate from it. The color variations and variegations will be described in a later part of the paper.

METHODS OF STUDY

The plants whose pericarp colors were to be studied were first crossed with plants having colorless pericarp and red cob. The crosses were made

by using pollen from the plants having the gene for colorless pericarp and red cob. The F_1 plants from such crosses have colored pericarp and red cob, because the colored pericarp of the female parent is dominant over the colorless pericarp of the male parent and the red cob of the male parent is dominant over the white cob of the female parent.

These F_1 plants were then back-crossed with strains having colorless pericarp and white cob. The progenies from such back-crosses consist of ears with colorless pericarp and red cob and ears with colored pericarp and white cob in approximately equal numbers. The ears with colorless pericarp and red cob represent the pericarp gene which was introduced in the original cross and are discarded. The ears with the colored pericarp and white cob, together with an occasional self-red or variegated ear, represent the gene that is being studied. Such ears have the gene for pericarp and cob color associated with the gene for colorless pericarp and white cob. By thus associating the inconstant genes that are being studied with stable genes which produce no color in the pericarp it is possible to eliminate the genes that are introduced in the various crosses, and to detect changes undergone by the gene under observation. For example, a strain with orange pericarp and white cob crossed with a strain with colorless pericarp and red cob will give F_1 plants with orange pericarp and red cob, of the constitution $\frac{OW}{WR}$. Such plants back-crossed with plants having colorless pericarp and white cob give the following two kinds of ears in approximately equal numbers: (1) Ears with colorless pericarp and red cob, of the constitution $\frac{WR}{WW}$. By discarding all ears with colorless pericarp and red cob the *WR* gene, which was associated with the *OW* gene in the first cross, was eliminated. (2) Ears with orange pericarp and white cob of the constitution $\frac{OW}{WW}$. Ears with variegated pericarp, and ears with red pericarp are regarded as being due to modifications of the *OW* gene.

The ears of each progeny were first studied for color intensity, variegation pattern, color changes, and the like, and grouped accordingly. Each ear was examined for color or pattern changes extending over more than a single kernel. The kernels were then shelled off the cob and each kernel was examined separately and classified as shown in the tables.

ACKNOWLEDGEMENTS

The writer is indebted to Doctors R. A. EMERSON and E. G. ANDERSON for the strain of orange-pericarp and white-cob maize used in this study, and for valuable suggestions as to the possible relationships of the various variegated patterns. Valuable assistance has also been rendered by Mr. GEORGE KLINE in making the drawings for figure *C*, plate 1, and in coloring the photographs for figures *A* and *B*, plate 1, and by Mr. J. F. BARHAM in making all of the photographs in the accompanying figures.

INCONSTANCY OF ORANGE PERICARP

The pericarp color which has been given the descriptive term "orange" is in fact a dilute red which is highly inconstant. It not only varies in intensity of color, but changes to colorless, to red, and to a number of distinct types of variegation.

Variation in color intensity of orange pericarp

Since the ears with orange pericarp and white cob in each back-cross progeny came originally from the same gene, a certain uniformity in color intensity was expected. This expectation was not fulfilled. When orange kernels of a certain color intensity are planted progenies are produced which vary in color from almost colorless to deep cherry red. In order to make a classification of these ears, arbitrary classes of color intensity were established, ranging from colorless, class A, to cherry red, class J. In figure *B*, plate 1, is illustrated a series of ears representing color-intensity classes, from class B, with a slight tinge of color, to class I, which is red.

Plantings were made from pedigreed ears of the various color-intensity classes with the results recorded in table 1. The progenies consisted of ears with colorless pericarp and red cob and ears with orange pericarp and white cob in approximately equal numbers. Occasional ears are found with variegated pericarp and variegated cob. In all cases the orange ears varied in color intensity around the color intensity of the parent ear as the modal class. When these ears were grouped in the arbitrary color-intensity classes there resulted distributions which approach that of a normal or skewed curve. Even the lightest ears are capable of producing progenies which range in color from almost colorless to deep cherry red. That these color variations have a genetic basis is well shown by the ear illustrated in figure 1 (page 381). This ear has two shades of red which are sharply delimited. The orange kernels from this ear produced plants with various shades of orange pericarp, typical of this strain, while the red kernels produced plants with dark-red ears.

COLOR CHANGES IN ORANGE PERICARP

The orange color of maize pericarp is due apparently to a reduced amount of pigment which is uniformly distributed throughout the pericarp tissue. Variations in the amount of pigment produce colors of different intensities. A striking feature of orange pericarp is the separation into its component colors, red and white (colorless), as may be seen on the ear illustrated in figure 2 and the kernels illustrated in figure *C*, plate 1. The orange may also change to darker orange and red, as well as to lighter orange and colorless, with no apparent production of the other component color. The ears of the different color-intensity classes were studied separately with the results given in tables 2, 3 and 4. In recording these data a special effort was made to get an accurate classification of the changes from orange to variegation. A kernel with a streak of colorless adjacent to a streak of red pericarp was classes as having a "line" of variegation. Since changes from orange to colorless occur very rarely, most of the apparently colorless areas represent changes to variegation. The data on changes from orange to red are accurate for areas which covered one-eighth of a kernel and larger areas. It will be noted that color changes of small size occur very frequently, while those large enough to cover more than a single kernel are exceedingly rare.

The orange ears were first studied for color changes extending over more than a single kernel and only 17 such changes were found in the two lots of ears studied. Most of these large changes were neither red, colorless, nor variegated, but dark-crown (a non-inherited pattern described by EMERSON (1917). Twelve of the large patches were changes to dark-crown, and 5 were changes to red. No change to variegation covered more than a single kernel. The kernels were then shelled off the ear and studied individually and 42,622 color changes of various kinds were recorded. Of these only 54 covered an entire kernel, 57 extended over approximately three-fourths of a kernel, 117 were as large as a half kernel, 254 covered one-fourth of a kernel, 1,222 were as large as one-eighth of a kernel, and 40,481 were smaller in size. If the size of color change is taken as an index of the stage in development at which the change occurred, these data indicate that the gene for orange pericarp persists until a later stage in development, when it may lose its stability, due possibly to changing conditions in the protoplasm as the plant approaches maturity, or due to a mechanical segregation of pigment-producing and non-pigment-producing *gene elements* as will be described later. Such gene changes are to be expected to occur more frequently in later stages of development because

LEGEND FOR PLATE 1

FIGURE *A*.—The ear with orange pericarp from which all the material used in this study originated.

FIGURE *B*.—A series of ears showing the variation in color intensity of orange pericarp.

FIGURE *C*.—Orange kernels with equal and adjacent segments of red and white (colorless). A variegation results when this process extends over the entire surface of the kernel. The intensity of the red segments varies with the intensity of the orange of the kernel.

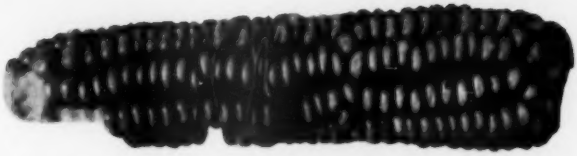


FIGURE *A*

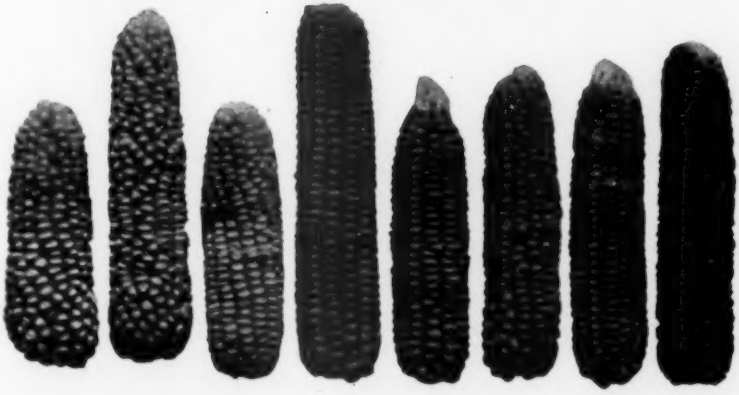


FIGURE *B*

FIGURE *C*

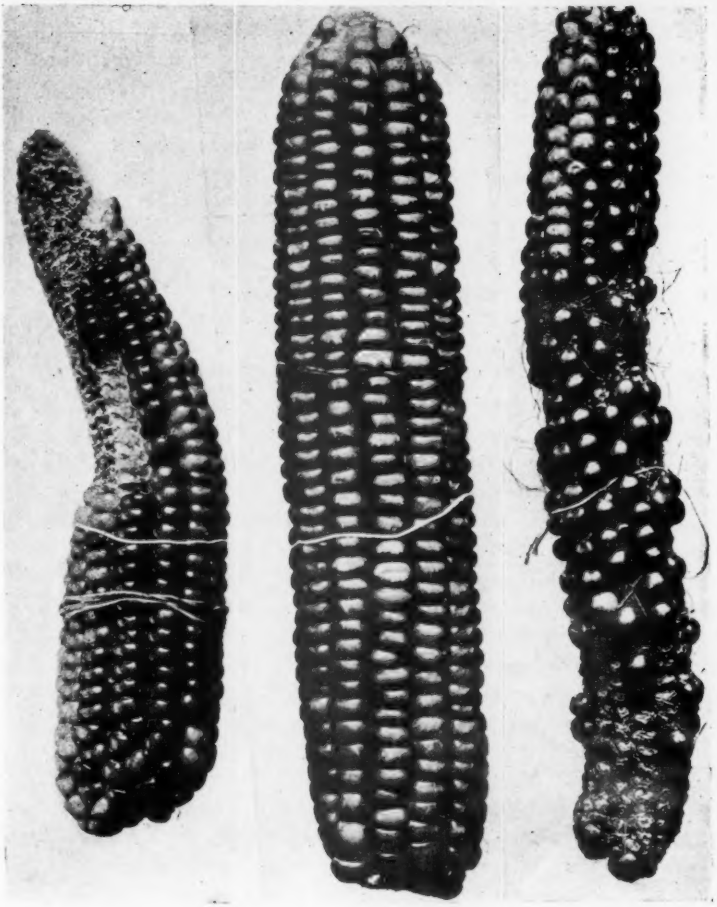


FIG. 1 FIG. 2 FIG. 3

FIGURE 1.—A light-orange ear with a large patch of its surface changed to deep orange or red. This is evidence that such changes have a genetic basis and are due to a change in the gene concerned.

FIGURE 2.—An ear with medium-orange pericarp showing the breaking up of the orange color into adjacent segments of red and white (colorless).

FIGURE 3.—An orange ear with a large patch of its surface changed to a typical variegation. This shows that orange and variegated pericarp are expressions of changed conditions in the same gene.

of the increased number of cells, but the rate of change is accelerated in late stages of development far beyond the expectancy for the increased number of cells. A significant characteristic of orange pericarp is that it persists until a late stage in development when it breaks up into its component colors, red and white (colorless), but growth stops before the segregation in the somatic cells is completed so that a large part of the pericarp remains orange self-colored.

RELATION OF COLOR INTENSITY TO RATE AND DIRECTION OF COLOR CHANGES

Orange pericarp of different color intensities changes to red, colorless and variegations, but with different frequencies.

Frequency of changes from orange to red

To get a measure of the rate of the color changes from orange to red only those that were accurately classified can be used. As has already been mentioned only the changes to red which extended over approximately one-eighth or more of a kernel were accurately recorded.

In table 2 are recorded the color changes from orange to deeper orange and red. In family 1504 the number of changes in orange pericarp of different intensities varied from 6 to 12 per thousand kernels studied. In the 41,564 kernels observed, 337 changes covering one-eighth of the surface of a single kernel, or larger areas, were recorded. This would be a frequency of 8.2 changes to red per thousand kernels. In family 1525 the changes to darker orange and red were more frequent, varying in orange pericarp of different intensities from no changes to 36 changes per thousand kernels observed. A total of 260 changes to red covering one-eighth of the surface of a kernel, or larger areas, were found in the 15,603 kernels studied. This is a frequency of 17 changes to red per thousand kernels.

Considering the two families together there was a total of 597 changes to red covering one-eighth of a kernel, or larger areas, in the 57,167 kernels observed. This is an average frequency of change from orange to red of about 10 per thousand kernels. Only 5 of the observed changes to red extended over more than a single kernel, 17 were as large as a single kernel, and the remainder were of smaller size. These observations indicate that pericarp color-changes from orange to red are infrequent until late in development, when they occur with a relatively high frequency. In general it may be said the frequency of change from orange to red increases with the color intensity of the orange pericarp.

In table 2 also are given the frequencies of changes to dark crown. In family 1504 the number of such changes in the orange pericarp of different

intensities varied from 12 to 42 per thousand kernels observed. In the 41,564 kernels studied there were 887 changes to dark crown recorded. This is a frequency of 20 dark crowns per thousand kernels. In family 1525 the number of dark crowns varied from 7 to 20 per thousand kernels, with an average frequency of 13 per thousand kernels. In the two families studied there was a total of 1088 dark crowns observed in the 57,167 kernels studied. This would be an average frequency of about 19 changes to dark crown per thousand kernels.

Frequency of changes from orange to lighter orange, light variegations or colorless

It was not possible to classify accurately the changes from orange to lighter colors. When small areas are concerned it is not possible to distinguish between colorless and variegations. In table 3 are listed all changes from orange to lighter colors. There was a total of 407 such changes in the 57,167 kernels observed, or about 7 in every thousand kernels. In general the frequencies of the changes to lighter colors vary inversely as the color intensity of the orange pericarp.

A record was also made of the changes to light crown. A total of 1234 kernels were partly or fully light-crowned. This is a frequency of 22 light crowns per thousand kernels as compared with 19 dark crowns per thousand kernels.

Frequency of changes from orange to variegation

The classification of the changes from orange to variegation was made with sufficient accuracy that all of the classes can be used in obtaining a measure of the frequency of the changes.

In table 4 are listed the changes from orange to variegation for the different color-intensity classes of families 1504 and 1525. There was a total of 14,371 changes from orange to variegation in the 57,167 kernels studied. The frequency of changes varies from 42 to 781 per thousand kernels. It is an interesting fact that variegations occur with a higher frequency in the classes of medium intensity of orange. No variegated areas covering more than a single kernel were found on the ears of families 1504 and 1525. Only 8 variegations covering as much as a kernel were found. Of the 14,317 variegations recorded 761 covered one-eighth or more of the surface of a kernel, while 13,556 were smaller in size.

The 761 variegations covering one-eighth or more of a kernel give a frequency of about 13 changes from orange to variegation per thousand

kernels. Equivalent changes from orange to red occurred with a frequency of about 10 per thousand kernels.

If the size of a color change may be taken as an index of the stage in development when the change occurred, the data in table 4 show definitely that the orange pericarp persists until late in development, when it changes to variegation with a high frequency.

The illustration in figure 3 shows a change from orange to variegation that extended over a number of kernels. Changes that affect so large an area are exceedingly rare.

TYPES OF VARIEGATION ORIGINATING FROM ORANGE PERICARP

Orange pericarp gives rise to a number of distinct variegated patterns. These range from a type that is exceedingly light, often having a single splash of color on an entire ear, to a very heavily variegated type with much color in the form of heavy lines and bands, and with occasional patches of self-red and dark-crown extending over a number of kernels. There seem to be two fairly well defined series of patterns which, for descriptive purposes, may be called (1) *light* variegations, with markings in the form of splashes, lines, and segments that are well dispersed, and (2) *heavy* variegations, in which the surface of the kernels is thickly covered with color markings.

In figure 4 are shown ears representative of the light-variegation patterns. Ears A and B represent a light variegation with a very coarse pattern. This pattern is distinctive in that the markings are in the form of broad lines or bands, with the remainder of the surface quite free from color markings. Ear C has an intermediate type of light variegation. A number of kernels have large segments or the entire surface colored while many kernels have smaller color markings. The variegated type represented by ear C is different from all other variegations in that the color markings are pink instead of red. The ear E represents an extremely light type of variegation, sometimes with only a single splash of color on an entire ear.

Representative types of heavily variegated patterns are illustrated in figure 5. Ear A has a very coarse pattern, made up of heavy lines and bands of color. Ears B and C represent an intermediate type of heavy variegation, with many lines and splashes of color, but without the coarse bands. The last ears, D and E, are typical of a very finely variegated type. The kernels are heavily marked but all the markings are in the form of fine splashes and lines uniformly distributed over the surface of the kernel.

INHERITANCE OF THE DIFFERENT VARIEGATION PATTERNS

Progenies were grown from each of the ears illustrated in figures 4 and 5 in order to determine the inheritance of the various patterns. The results of these tests are given in table 5.

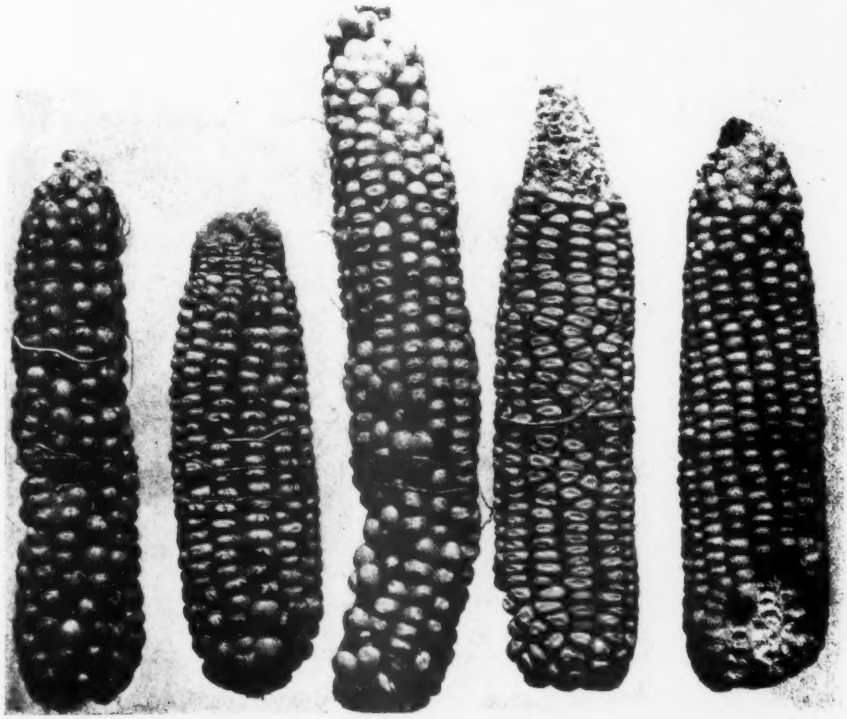


FIGURE 4.—A series of ears having a light pattern of variegation ranging from ears with many bands and lines of red (ear A) to ears that have often only a single splash of red color (ear E).

Inheritance of the light-variegation patterns

The parent plant of ear B, figure 4, had the gene for variegation associated with a gene for red cob and colorless pericarp and was back-crossed with a plant homozygous for white cob and colorless pericarp. The progeny consisted of 25 ears with red cob and colorless pericarp and 22 ears that

were variegated. Three of the variegated ears were coarser in pattern than the parent ear, 16 ears had a pattern quite similar to the parent ear, 2 ears had a distinctly finer pattern and one ear had only a few splashes of color on the entire ear.

The ear C, figure 4, was produced on a plant grown from one of the variegated kernels of the ear illustrated in figure 3. It had a gene for variegated pericarp associated with a gene for red cob and colorless pericarp and was back-crossed with a plant homozygous for white cob and colorless pericarp. The progeny consisted of 70 ears with red cob and colorless pericarp and 66 variegated ears. Three of the variegated ears

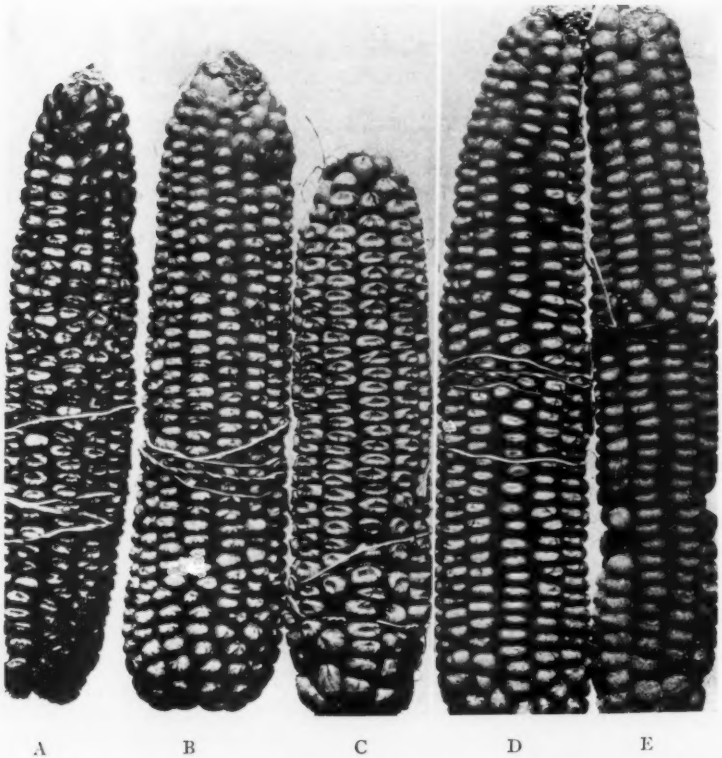


FIGURE 5.—A series of ears having a heavy pattern of variegation, ranging from ears with heavy bands and lines of red (ears A and B) to ears with many fine lines and splashes of red uniformly distributed over the kernels (ears D and E).

had a coarse pattern, like ear B, figure 4, 62 ears had the pattern of the parent ear, and one ear had a light pattern like that of ear D, figure 4.

Ear D, figure 4, was open-pollinated and produced a progeny with red cob and colorless pericarp, red cob and red pericarp, and variegated pericarp. All of the variegated ears were of the pattern of the parent ear.

Inheritance of the heavy-variegation patterns

Ears A and B in figure 5 were open-pollinated. A large percentage of the variegated ears of their progenies had the variegation patterns of the parent ears, but ears with medium and fine patterns were also found.

The plant that produced ear C, figure 5, was heterozygous for red cob and colorless pericarp and variegated cob and variegated pericarp and was back-crossed with a plant homozygous for white cob and colorless pericarp. The progeny consisted of 55 ears with red cob and colorless pericarp, 52 ears with variegated cob and variegated pericarp, and one ear with red cob and red pericarp. Thirty-five of the variegated ears had the pattern of the parent ear, 15 ears had a coarser pattern, 11 being like ear B, figure 5, and 4 like ear A, figure 5, and 2 ears had the very fine pattern of ear E, figure 5.

The parent plant of ear D, figure 5, was also heterozygous for variegated cob and pericarp and for red cob and colorless pericarp and was back-crossed with a plant homozygous for white cob and colorless pericarp. The resulting progeny consisted of 36 ears with red cob and colorless pericarp and 37 ears with variegated cob and variegated pericarp. Seventeen of the variegated ears had the fine pattern of the parent ear, 15 ears had a medium pattern much like C, figure 5, and 5 ears had a pattern as coarse as that of ear B, figure 5.

The heavy variegations form a continuous series ranging from very coarse to very fine patterns and cannot be grouped into distinct types. That these variegation patterns have a genetic

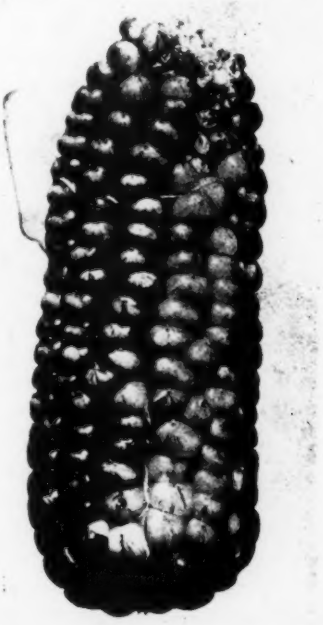


FIGURE 6.—An ear with two patterns of variegation, showing that pattern differences are due to changes in the gene.

basis is shown by the occurrence of more than one pattern on the same ear, as may be seen from the illustration in figure 6. Whenever two variegation patterns occur on the same ear they are sharply delimited and evidently represent a change in the variegation gene.

The progenies from both the light and heavy variegations include a number of distinct patterns in the form of a frequency curve, with the pattern of the parent ear as the modal class. In this respect the variegations are like the orange pericarps from which they originated.

In the progeny of every variegated ear tested, ears with red cob and colorless pericarp and ears with variegated cob and variegated pericarp occurred in approximately equal numbers, thus indicating that a single pericarp gene was concerned in the production of each variegation. The variation in the pattern of variegation in each progeny is thought to be due to changes in the variegation gene.

RELATION BETWEEN COLOR INTENSITY OF ORANGE PERICARP AND TYPE OF VARIEGATION

In order to determine the relation between color intensity of orange pericarp and type of variegation several bushels of orange ears were grouped in five color-intensity classes ranging from very light orange to red. All kernels that had enough of the surface variegated to be classified were taken from each group of ears and classed as light variegations or heavy variegations, with the results given in table 6. The very light orange ears produced only light variegations, while the red ears produced nothing but heavy variegations. The number of heavy variegations increases with the intensity of orange pericarp. These results show that there is a definite correlation between the intensity of orange color and the types of variegation that are produced.

Color changes in the variegated patterns

The variegated patterns undergo somatic color changes to dark crown, red orange, colorless, and to other patterns of variegation.

Frequency of changes in light variegation

The ears of the progenies grown from the ears B, C and D, in figure 4, were studied for color changes with the results recorded in table 7. A total of 3181 kernels were observed in the strain having a fine pattern. There were 7 kernels with one-eighth or more of their surface changed to self-red, or approximately 2 such changed kernels for every thousand kernels observed. Also 30 kernels were partly or fully dark-crowned, giving a

frequency of 9.43 such changes per thousand kernels observed. No color change was observed that covered more than a single kernel.

The strain with a medium pattern of light variegation, the progeny of ear C, figure 7, is different from other variegations in that the color is deep pink instead of red. The number of kernels observed was 9286. Of these, 198 kernels had one-eighth or more of the surface pink self-colored. This would be a frequency of change to pink color of 21.28 per thousand kernels observed. The frequency of change to self-color was about ten times as great in the medium as in the fine pattern, the latter having seven kernels with one-eighth or more of their surface changed to red, giving a frequency of change of 0.75 kernels per thousand studied. There were 74 changes to dark crown covering one-fourth of a kernel or larger areas, which is a frequency of 8 changes per thousand kernels observed in the medium pattern, as compared with 9.43 for the fine pattern.

Changes to self-color and dark crown occur much more frequently in the coarse pattern than in the medium and fine patterns. Among the 2966 kernels from coarsely variegated ears, were found 258 changes to red affecting one-eighth or more of a kernel, and 117 changes to dark crown. This would be one change to red for every 11.5 kernels, or 87 changes for every thousand kernels studied, and one change to dark crown for every 25.4 kernels, or 40 per thousand. Changes to self-color occur 40 times as frequently in the coarse pattern as in the fine and 4 times as often in the medium pattern. The changes to dark crown occur about 5 times as often in the coarse pattern as in the medium and fine patterns.

Frequency of changes in heavy variegation

The ears with heavy variegation patterns were separated into groups having coarse, medium and fine patterns, respectively. The ears of each of these groups were studied for color changes to self-color with the results given in table 8. Only areas covering one-fourth or more of a single kernel were recorded.

The ears with a coarse variegation pattern yielded a total of 86,550 kernels. There were 48 changes to red that extended over more than a single kernel; 225 changes were as large as a single kernel, or 2.6 self-red kernels per thousand kernels studied. Color changes affecting smaller areas are more and more frequent as is shown in table 8. There were 1978 color changes affecting one-fourth of a kernel, which is a frequency of 23 changes per thousand kernels. Considering all changes to self-color covering one-fourth of a kernel or larger areas, there was a total of 3840 in the

86,550 kernels observed. This is a frequency of 44 changes per thousand kernels from the coarsely variegated ears.

There was a total of 18,427 kernels taken from the ears with a medium-coarse variegation pattern. Only two changes to self-red covering more than a single kernel were found. There were 7 color changes covering a single kernel which is a frequency of less than 0.4 kernel per thousand kernels observed. Forty-six kernels had one-half of the surface self-colored and 69 kernels had approximately one-fourth of the surface self-colored, with frequencies equal to 2.5 and 3.7 changes per thousand kernels, respectively.

Among the 18,427 medium variegated kernels there was a total of 131 changes to self-color covering one-fourth of a kernel or larger areas. This would be an average frequency of about 7 changes to self-color per thousand kernels, as compared with 44 such changes in the coarsely variegated patterns.

There was a total of 7560 kernels from the finely variegated ears. No color changes extending over more than a single kernel were found. There were 2 self-red kernels, 6 kernels with half the surface red, and 11 kernels with approximately one-fourth of the surface red. Among the 7560 kernels were found a total of 20 changes to red covering one-fourth of a kernel or larger areas. This is equal to an average frequency of 2.6 changes per thousand kernels.

From these results it is evident that changes to self-color increase with the coarseness of the heavily variegated pattern.

Frequency of changes to dark crown in the variegated types

A record was kept of the changes to dark crown in the variegated kernels studied, with the results given in table 9. Of the 86,550 kernels taken from heavy coarsely variegated ears 2739 kernels were recorded as partly dark-crowned, 870 kernels were fully dark-crowned. There were 822 dark-crown patches that extended over more than a single kernel. This would be a total of 4431 dark-crown changes, which is a frequency of about 51 changes per thousand kernels. In the heavy medium variegations 891 changes to dark crown covered a part of a kernel, 142 kernels were fully dark-crowned, and 66 dark-crown changes extended over more than a single kernel. In all there were 1073 changes to dark crown in the 18,427 kernels observed. This a frequency of approximately 58 changes per thousand kernels. In the heavy fine variegation, of the 7566 kernels studied, 300 kernels were partly dark-crowned, 81 were fully dark-crowned and 33 changes to dark crown extended over more than a single kernel.

The total of 414 dark-crown changes in the 7560 finely variegated kernels is a frequency of 55 per thousand kernels. A summary of the changes to dark crown in the variegated patterns follows:

Types of variegation	*Number of dark-crown per 1000 kernels studied*
Heavy coarse,	51.0
Heavy medium,	58.0
Heavy fine,	55.0
Light coarse,	40.0
Light medium,	8.0
Light fine,	9.5

In the heavy variegations dark crowns occurred with about the same frequency in all the types. In the light variegations dark crowns occurred about as frequently in the fine type as in the medium type, but in the coarse type the frequency was five times as high.

Frequency of changes from heavy to light variegations

All the heavy types of variegation studied change to colorless or light-variegated types. Since colorless types originate from the heavy variegations only rarely, it may be assumed that the apparently colorless areas are in reality light variegations. The frequency of change from heavy variegation to light variegation as observed in three lots of ears is given in table 10. In the coarse pattern there was an average of about 8 changes to light variegation per thousand kernels, while in the medium pattern there were approximately two light variegations per thousand kernels. Only changes affecting one-fourth of a kernel or larger areas were recorded.

Reverse changes from variegation to orange pericarp

The variegated types revert occasionally to orange pericarp. While the patches that cover one-fourth of a kernel or larger areas can be distinguished from red easily, the smaller areas are more difficult to classify accurately. Only a few such reverse changes have been observed. In family 1501 were found 2 kernels with one-half of the surface changed to orange, and one kernel with one-fourth of the surface orange-red in a total of 40,975 kernels. Among the medium-variegated kernels of family 5492 one kernel was found with about one-sixteenth of its surface changed to orange in the total of 11,732 kernels observed. Among the 866 finely variegated kernels of family 5492 were found 17 kernels with about one-sixteenth of the surface changed to orange.

Changes from pink variegation to red variegation

In typical variegations in maize pericarp the contrasting colors are white (colorless) and red. In family 5497, as already mentioned, the

colored areas of the variegated kernels are light pink instead of red. This pink type of variegation gives rise to the typical variegation with red-pigmented areas. Of the 9286 pink-variegated kernels studied 7 had red variegations which evidently represent independent changes from the pink type. This would be a frequency of nearly 0.8 changes per thousand kernels.

COLOR CHANGES IN THE CHERRY-RED PERICARP THAT ORIGINATED FROM ORANGE PERICARP

The deep-red pericarps that originate from orange pericarp are fairly constant, but reverse changes do occur as indicated below.

Reverse changes from cherry-red to orange

A large number of ears with cherry-red pericarp that originated from strains having orange pericarp, having a total of 32,568 kernels, were studied for color changes. The kernels were shelled off the ears and examined individually for color changes. The most frequent color change was the reverse change to orange. One kernel was one-half orange, 2 kernels were one-fourth orange, 48 kernels had a band of orange color, and 247 kernels had a fine line of orange color. Excluding the kernels that had only a fine line of orange pericarp, the frequency of change from red to orange was about 1.6 per thousand kernels. Also 71 kernels had a part of the crown changed to orange and 8 kernels were fully orange-crowned.

Reverse changes from cherry-red to variegations

The changes from cherry-red to colorless or variegation were less frequent than the changes to orange. In the 32,568 kernels studied the largest color change to variegation covered approximately one-fourth of a kernel, and only one change as large as this was found. There were 8 kernels with a narrow band of colorless pericarp and 16 kernels with a fine line of colorless pericarp. Excluding the kernels with only a fine line of colorless pericarp, the frequency of change from red to variegation was about one change in three thousand kernels.

There were also a few changes to light crown. One kernel was entirely light-crowned, and 20 kernels were partly light-crowned.

Stability of colorless pericarp originating from orange

A large number of ears with colorless pericarp, derived from the orange through the variegated patterns, were examined for color changes, but no such changes have yet been observed. The kernels were shelled off the ears and examined individually. From these studies it may be concluded

that the gene for colorless pericarp which originates from the orange-pericarp gene has the stability of the ordinary white-cob colorless-pericarp gene.

Summary of color changes in the orange-pericarp series

A summary of the various changes, including the direction and frequency of changes, is given in figure 7. The most frequent changes are from orange to variegation (13 per thousand kernels) and from orange to red (ten per thousand kernels). The heavy variegations also appear to be quite unstable, as they change to lighter variegations with a fairly high frequency (eight per thousand kernels). Changes from red to orange, from red to variegation, and from variegation to orange occur with a low frequency, ranging from 0.3 and 1.6 changes per thousand kernels observed.

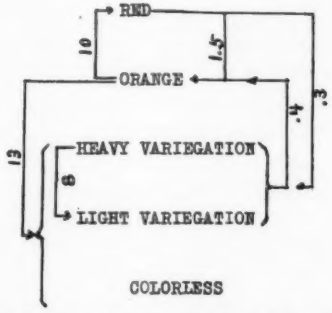


FIGURE 7.—A diagram to show the relative frequencies of pericarp-color and pattern changes expressed in numbers per thousand kernels.

INTERPRETATION AND DISCUSSION

Any interpretation of the pericarp-color and pattern changes described on the foregoing pages must account for the following facts: (1) Orange pericarp is inconstant; it produces progenies that vary in color intensity from a slight tinge of color to deep cherry-red, in the form of a frequency curve. (2) The orange color separates into its component colors, red and white (colorless), to form a variegation. Variegations seem to occur more frequently in the orange pericarp of medium intensity than in the extremely light or extremely dark classes. (3) The variegations are inconstant, varying in pattern from fine to coarse in the form of a frequency distribution. Some patterns are *light*, with few well-dispersed markings, while other patterns are *heavy*, made up of many closely-set color markings. (4) There is a correlation between intensity of orange color and the type of variegation which originates from it. The percentage of heavy variega-

tions increases with the color intensity. (5) All color and pattern changes are reversible, but with different frequencies.

The nature of the gene

The gene is doubtless highly complex in its organization so that changes in its chemical composition or rearrangements of its structural parts occur more or less frequently in all genes. Whatever the nature of the genes may be, the work on Drosophila has shown that they occupy definite loci in the chromosomes. The most distinctive feature of the gene, as pointed out by MULLER (1922), is its property of self-propagation. In preparation for each mitosis each gene must reproduce itself by attracting to itself from the protoplasm materials of a similar kind which are then separated into the daughter genes. Gene constancy demands that the daughter genes be identical in chemical composition and in structural arrangement. That genes do change, and that they are capable of changing so as to produce a number of different effects is shown by the common occurrence of multiple allelomorphs. In most cases the changes are quantitative so as to produce a linear series of effects.

The changes or variations in the gene for orange pericarp form an orthogenetic series of colors ranging from whitish to deep cherry-red. Such a quantitative variation can be most easily explained by assuming that the gene for orange pericarp is a compound structure, made up of pigment-producing *gene elements* and non-pigment-producing *gene elements*. According to this view the intensity of color depends upon the relative numbers of the contrasting gene elements incorporated in the structure of the gene. An excess of non-pigment-producing gene elements would produce a light orange color, while an excess of the pigment-producing gene elements would produce a deep orange or red color.

It has been found that orange pericarp of medium intensity produces a progeny ranging from nearly colorless to dark orange or red in a distribution which approaches that of a normal curve, the modal class being medium orange like the ear from which the planting was made. This distribution can be skewed to the light or dark end of the color range by planting from a light-orange ear or a dark-orange ear, respectively. These results would obtain if the gene elements are self-propagating and are divided by random assortment, at each mitosis, into the daughter genes. DE VRIES's observations indicate that color intensity in Hesperis behaves in inheritance like the corresponding color variations in maize. The seeds of whitish plants produced progenies that were predominately whitish-flowered, but some lilac-flowered plants and a few violet-flowered

plants also appeared. The lilac-flowered plants, which correspond to the medium orange colors in maize, produced offspring with the whole range of color from whitish to violet. Only 5 violet-flowered plants were tested and they produced violet-flowered offspring.

Evidently the various colors in the pericarp of maize and in the petals of Hesperis are the expressions of genes that are made up of definite numbers of pigment-producing and non-pigment-producing gene elements. Each gene must reproduce itself between every two mitoses, and many mitoses occur between the fertilized egg and the formation of the tissues in which the color is expressed. Because of the random assortment of gene elements the existing relation between the contrasting gene elements would be maintained in a relatively large number of plants. In a somewhat smaller number of plants there would be expected a slight excess of the pigment-producing or non-pigment-producing elements in the tissue in which the color is expressed, due to slight inequalities in the separation of the elements at the mitoses, or to a slight difference in the rate of reproduction of the individual gene elements. In the same manner a larger excess of one or the other kind of gene element would be expected in a smaller number of plants, and so on. The progeny of an individual with a gene behaving in development as indicated above would consist of a series of individuals ranging from very light colors, due to a gene with an excess of gene elements for no color, to deep colors, due to a gene with an excess of color-producing gene elements.

A similar quantitative expression of color would result in the presence of multiple modifying factors similar to the modifiers of eosin eye color in Drosophila, reported by BRIDGES (1919). Suitable studies to determine whether or not modifying factors influence color intensity in orange pericarp have not been made, but changes from medium orange to full red, as illustrated in figure 1, would hardly be expected if a large number of modifying factors are concerned.

The most striking as well as the most significant feature of orange pericarp is that it separates into its component colors, red and white (colorless). It will be recalled that in Hesperis a similar separation of colors occurs. In maize pericarp the replacement of orange by adjacent and equal segments of red and white, as illustrated in figure *C*, plate 1, may be considered good evidence that the gene which had been producing the orange color divided into daughter genes in such a way that one of them included all of the pigment-producing gene elements while the other lacked them entirely. The illustrations in figure *C*, plate 1, represent kernels from orange ears of different intensities. The adjacent red and white seg-

ments represent the cell progenies of the daughter genes with and without pigment-producing gene elements, respectively. In orange pericarp the medium colors separate into red and white with a higher frequency than the lighter and darker colors. Apparently, the same thing is true of Hesperis, as DE VRIES (1910) described the lilac-colored flowers as being often striped or spotted. The fact that color segregation in the somatic tissues of both maize and Hesperis occurs most frequently in the colors of average intensity indicates a relationship between the relative numbers of the two kinds of gene elements in the structure of the gene and its stability. Like gene elements are mutually attractive while the unlike ones are mutually repulsive. A gene with only one kind of element in its structure, as the one for colorless pericarp, should be compact, due to the mutual attraction of the like elements, and therefore highly stable. Similarly a gene with unlike elements in its structure, as the gene for orange or variegated pericarp, would tend to be dispersed and its structure would be the resultant of the unlike genes in a state of equilibrium with varying stability.

EMERSON (1922) made the important observation that changes to self-red occur somewhat more frequently in heterozygous than in homozygous variegated ears, and concluded that a gene for variegation is more mutable when associated with a gene for colorless pericarp than when it is present in the duplex condition. It will do no harm, it is hoped, to offer an interpretation of this observation based on the gene structure discussed above. The higher frequency of change to self-red in pericarp tissue which has a gene for variegation associated with a gene for colorless may be due simply to an accumulation of pigment-producing gene elements. Any accumulation of a particular kind of element would be retained if there is no interchange of parts between the highly stable colorless-pericarp gene and the variegated gene with which it is associated. In homozygous variegated pericarp the associated genes are made up of similar structural units. If these genes which are unstable because they are made up of contrasting gene elements, interchange parts between mitoses any accumulation of one kind of gene element in one of the genes might be negatived by an accumulation of the contrasting gene element in the homologous gene, so that the actual number of changes to red would be less than in pericarp tissue where the unstable variegation gene is associated with a highly stable gene like the one for colorless pericarp between which there is no interchanging of parts.

An inconstant gene, like the one responsible for orange pericarp in maize or the one that causes lilac color to develop in the petals of Hesperis may be due to a single chemical change of such a character as to change the

nature of one of the structural elements of the gene. This change followed by a random assortment of the gene elements would provide the mechanism for the quantitative expression of color and for the occurrence of variegations in the tissues of the pericarp of maize and of the petals of Hesperis.

The nature of variegation

The equal and adjacent segments of red and colorless tissue on otherwise orange-colored kernels represent early stages in the production of a variegation. In such kernels the separation of the orange into red and white began too late in development of the pericarp to affect more than a small segment of the tissue. When this separation begins earlier in the development of the pericarp tissue the variegation extends over larger segments, a whole kernel, or a number of kernels.

The fundamental difference between orange pericarp and variegated pericarp is that in the former the pigment is diffused throughout the tissue, while in the latter the pigment is limited to segments and patches of various sizes. In the gene for orange pericarp the complete segregation of unlike gene elements usually occurs not at all, or only very late in development, so that only small segments of red and colorless tissues are produced. In the gene for variegation the segregation of unlike gene elements begins early enough so that they are completely segregated by the end of pericarp-tissue development.

There is a decided correlation between the intensity of orange color and the type of variegation that originates from orange pericarp. Practically all of the variegations that spring from the light orange colors are very light in pattern. The percent of heavy variegations increases with the color intensity of the orange from which they originate. These variegations are not constant in inheritance, but produce progenies that vary in pattern from light to heavy and from fine to coarse. The results are quantitative, quite like the variation in intensity of the orange color from which these variegations sprang. Evidently, the light variegations are produced by genes with a relatively small number of pigment-producing elements, while the heavy variegations are produced when the genes have an excess of pigment-producing gene elements.

It seems evident that a color variegation is produced when a gene includes both pigment-producing and non-pigment-producing *gene elements* which become segregated in the somatic tissue, in the course of development, by the mechanism of mitosis.

SUMMARY

The important features of the present paper may be summarized as follows:

1. Orange pericarp in maize varies in color from whitish to deep cherry-red.

2. The progeny of an ear of medium-orange pericarp includes ears ranging in color from whitish to red in the form of a frequency curve. The curve may be skewed towards the light or dark end of the range by planting from ears that are light- or dark-orange, respectively.

3. The orange pericarp gives rise to variegations of a number of distinct types.

4. Light-orange ears produce variegations that are prevailingly light in pattern, while dark-orange ears tend to give rise to heavy variegations. Evidently, there is a correlation between color intensity of orange pericarp and variegation pattern. According to the interpretation the color intensity of the orange pericarp, and the number and size of the color-markings of the variegations increase with the number of pigment-producing gene elements in the pericarp gene.

5. All color and pattern changes are reversible, but with different frequencies.

6. The gene for orange pericarp is thought to be made up of pigment-producing and non-pigment-producing gene elements. Variations in the orange color are due to different proportions of the contrasting gene elements in the pericarp gene.

7. The occurrence of adjacent segments of red and white (colorless) of equal size, on an otherwise orange-colored kernel, indicates that the gene for orange pericarp divided in such a way that its pigment-producing elements were separated from its non-pigment-producing elements by the mechanism of mitosis.

8. Variegations are produced when the contrasting gene elements become completely segregated in the somatic tissues in the course of development. Since variegations in general, in animals as well as in plants, are fundamentally alike in their inconstancy and in the nature of their color changes, it may be concluded that all typical variegations are produced in a manner similar to that described above for maize pericarp.

LITERATURE CITED

BRIDGES, C. B., 1919 Specific modifiers of eosin eye color in *Drosophila melanogaster*. Jour. Exp. Zoöl. **28**: 337–384.

CORRENS, C., 1910 Der Übergang aus dem homozygotischen in einen heterozygotischen Zustand im selben Individuum bei buntblättrigen und gestreiftblühenden Mirabilis-Sippen. Ber. deutsch. bot. Gesell. **28**: 418–434.

EAST, E. M., and HAYES, H. K., 1911 Inheritnce in maize. Connecticut Exp. Sta. Bull. 167. Pp. 3–142.

EMERSON, R. A., 1911 Genetic correlation and spurious allelomorphism in maize. Nebraska Agric. Exp. Sta. Ann. Rpt. **24**: 59–90.

1913 The inheritance of a recurring somatic variation in variegated ears of maize. Amer. Nat. **48**: 87–115.

1917 Genetical studies of variegated pericarp in maize. Genetics **2**: 1–34.

1922 The origin of variations. Amer. Nat. **56**: 64–79.

GREGORY, R. P., 1911 Experiments with *Primula sinensis*. Jour. Genetics **1**: 74–132. *Pls. 30–32.*

HARTLEY, C. P., 1902 Improvement of corn by seed selection. United States Dept. Agric. Yearbook, **1902**: 539–552.

HAYES, H. K., 1917 Inheritance of a mosaic pericarp pattern color of maize. Genetics **2**: 261–281.

MULLER, H. J., 1922 Variations due to change in the individual gene. Amer. Nat. **56**: 32–50.

PUNNETT, R. C., 1922 On a case of patching in the flower color of the sweet pea (*Lathyrus odoratus*). Jour. Genetics **12**: 256–281.

VRIES, H. DE, 1905 Species and varieties: their origin by mutation. 2nd ed., xviii+847 pp. Chicago: Open Court Pub. Co.

1910 The mutation theory. Vol. 2. viii+683 pp. Chicago: Open Court Pub. Co.

Appendix—Tables 1 to 10

Table 1

Variation in color intensity of orange pericarp.

Pedigree	Color intensity of the orange kernels planted	Pericarp color of the progeny ears									
		Variegated	Colorless	Orange ears with intensities indicated below:							
				B	C	D	E	F	G	H	I
5485	B			16	13	4	2				
5532	B			11	10	3	1				
Total	B			27	23	7	3				
5533	C				10	2					
5534	C			5	38	6	3				
Total	C			5	48	8	3				
1504	D	1		7	31	50	35	9			
1506	D	1	2	5	16	35	7	1			
1512	D			4	28	77	37	5	3	1	
1521	D	1		4	11	36	18	4			
5535	D					11	4				
5536	D				2	25	2				
Total	D	3	2	20	88	234	103	19	3	1	
1519	E	2		1	3	11	41	8	1		
1520	E	1			1	13	30	11	4	2	
1524	E				9	25	17	7	3		
1525	E			3	14	32	17	10	4		
5537	E				1	6	96	4	1		
5539	E			2	2	3	50	3			
5486	E		1			2	26	4	1		
5487	E		1			8	43	1			
Total	E	3	2	6	30	100	320	48	14	2	
1522	F					6	16	38	18	1	1
5522	F						6	42	4	1	
5527	F				1	1	5	40	3	2	
Total	F				1	7	27	120	25	4	1
1510	G				1	5	11	23	49	15	3
3848	G						7	26	52	19	3
5529	G						1	6	54	2	
Total	G				1	5	19	55	155	36	6
5542	H								4	66	5
5543	H								10	63	12
Total	H								14	129	17
5544	I								1	2	50

TABLE 2

Frequencies of color changes in maize pericarp from orange to red.

PEDIGREE	INTENSITY OF ORANGE PERICARP	NUMBER OF KERNELS OBSERVED	CHANGES FROM ORANGE TO RED EXPRESSED IN KERNELS AS INDICATED IN COLUMN HEADS								DARK CROWN				
			¼	½	¾	¾	1	> 1	Total	Number per thousand	Part	Full	> 1	Total	Number per thousand
1504	B	2135	9	5	4	2	1	–	21	9.8	58	3	1	62	42
	C	9206	64	15	22	4	5	–	110	12.0	250	26	4	280	33
	D	13399	39	16	15	4	3	1	78	6.0	139	23	1	163	12
	E	14086	62	22	12	5	1	–	102	7.2	283	40	–	323	23
	F	2738	14	7	3	–	1	1	26	9.1	46	13	–	59	22
1525	C	1645	6	3	4	1	1	–	15	9.1	16	3	1	20	12
	D	3012	36	17	16	–	2	1	72	24.0	42	15	3	60	20
	E	3313	84	16	10	4	3	1	118	36.0	47	13	–	60	18
	F	5493	16	5	4	1	–	1	27	5.0	30	6	1	37	7
	G	1120	18	7	2	1	–	–	28	25.0	6	1	1	8	7
	H	1020	—	—	—	–	–	–	00	——	15	1	–	16	15
Total		57167	348	113	92	22	17	5	597	10.0	932	144	12	1088	19

TABLE 3

Frequencies of color changes in pericarp of maize from orange to lighter orange, light-variegated, or colorless.

PEDIGREE	INTENSTIY OF ORANGE PERICARP	NUMBER OF OF KERNELS OBSERVED	CHANGES TO LIGHTER ORANGE, LIGHT VARIEGATED OR COLORLESS IN KERNELS AS EXPRESSED IN COLUMN HEADS BELOW							LIGHT CROWN			
			¼	½	¾	¾	1	Total	Number per thousand	Part	Full	Total	Number per thousand
1504	B	2135	11	5	2	1	5	24	11	15	1	16	7
	C	9206	43	11	6	2	2	64	7	167	20	187	20
	D	13399	21	13	16	5	9	64	5	107	55	162	12
	E	14086	76	7	11	1	2	97	7	431	58	489	33
	F	2738	26	3	3	1	1	34	13	89	28	117	44
1525	C	1645	12	4	2	1	–	19	11	19	7	26	16
	D	3012	21	7	6	3	4	41	14	49	9	58	19
	E	3313	20	9	7	2	2	40	12	64	15	79	24
	F	5493	9	1	2	1	1	14	3	30	7	37	7
	G	1120	8	2	—	–	–	10	9	12	3	15	13
	H	1020	—	—	—	–	–	—	0	48	—	48	50
Total		57167	247	62	55	17	26	407	7	1031	203	1234	22

TABLE 4

Frequencies of color changes from orange to variegation in maize pericarp.

PEDIGREE	INTENSITY OF ORANGE PERICARP	NUMBER OF KERNELS OBSERVED	CHANGES EXPRESSED IN KERNELS AS INDICATED IN THE COLUMN HEADS							
			1/2	1/3	1/4	2/3	1/5	1	Total	Number per thousand
1504	B	2135	149						149	71.4
	C	9206	1600	2	2	1	—	—	1605	174.5
	D	13399	2304	7	7	8	4	2	2332	174.0
	E	14086	3398	95	11	6	9	1	3520	252.1
	F	2738	462	1	2	3	2	—	470	171.5
1525	C	1645	607	17	2	—	—	—	626	380.2
	D	3012	1655	99	17	3	—	2	1776	588.2
	E	3313	2261	287	28	6	1	—	2583	781.2
	F	5493	899	104	6	1	2	3	915	465.5
	G	1120	280	14	3	1	—	—	298	266.0
	H	1020	41	1	1	—	—	—	43	41.7
Total		57167	13656	627	79	29	18	8	14317	250.0

TABLE 5

Variation in type of variegation.

DESCRIPTION OF SEED PLANTED	PROGENY EARS							
	Light variegation				Heavy variegation			
	A	B	C	D	A	B	C	D
Light variegation (figure 4)								
1. Coarse pattern, ear B.....	3	16	2	1				
2. Medium pattern, ear C.....		3	62	1				
3. Fine pattern, ear D.....				All				
Heavy variegation (figure 5)								
1. Coarse pattern, ear B.....					12	41	1	
2. Medium pattern, ear C.....					4	11	35	2
3. Fine pattern, ear D.....					1	4	15	17

TABLE 6

Types of variegations originating from orange pericarp of different intensities of color.

DESCRIPTION OF ORANGE PERICARP	TYPES OF VARIEGATION	
	Percent light	Percent heavy
Very light orange.....	100.0	0.0
Light orange.....	80.6	19.4
Medium orange.....	67.0	33.0
Dark orange.....	57.5	42.5
Red.....	0.0	100.0

TABLE 7

Frequencies of changes from light variegation to red.

PEDIGREE	PATTERN	NUMBER OF KERNELS OBSERVED	CHANGES TO SELF-RED COVERING PARTS OF KERNELS AS INDICATED IN COLUMN HEADS							
			¼	½	¾	1	>1	Total	NUMBER per thousand
5495	Fine	3181	5	1	—	—	1	–	7	2
5497	Medium	9286	83	63	39	16	2	7	210	23
5498	Coarse	2966	156	49	33	12	5	3	258	87
Total		15433	244	113	72	28	8	10	475	31

TABLE 8

Frequencies of changes from variegation to self-color.

PEDIGREE	PATTERN OF VARIEGATION	NUMBER OF KERNELS OBSERVED	CHANGES FROM VARIEGATION TO SELF-COLOR EXPRESSED IN KERNELS AS INDICATED BELOW						
			¼	½	¾	1	> 1	Total	Number per thousand
1501	Coarse	40975	1129	590	238	139	31	2127	53
1502	Coarse	17777	264	200	76	21	4	565	32
1503	Coarse	21497	471	219	94	44	4	832	40
5489	Coarse	1587	21	30	9	8	2	70	44
5492	Coarse	1826	14	27	4	3	–	48	30
5493	Coarse	2888	79	82	20	10	7	198	66
Total	Coarse	86550	1978	1148	441	225	48	3840	44
1503	Medium	4074	19	6	0	1	—	26	6.4
5489	Medium	2621	22	17	5	2	—	46	17.5
5492	Medium	11732	28	23	2	4	2	59	5.0
Total	Medium	18427	69	46	7	7	2	131	7.0
5489	Fine	6700	11	6	1	2	—	20	3.0
5492	Fine	866	—	—	—	—	—	—	0.0
Total	Fine	7566	11	6	1	2	—	20	2.6
Total, all patterns		112543	2058	1200	449	234	50	3991	35.5

TABLE 9

Frequencies of changes to dark crown in the variegated types.

PEDIGREE	PATTERN	NUMBER OF KERNELS OBSERVED	DARK CROWN COVERING AREAS INDICATED BELOW				
			Part of kernel	Entire crown	Patch of more than one kernel	Total	Number per thousand
1501	Coarse	40975	1101	315	384	1800	44
1502	Coarse	17777	497	170	162	807	45
1503	Coarse	21497	730	277	216	1223	57
5489	Coarse	1587	133	29	20	182	114
5492	Coarse	1826	109	18	13	140	77
5493	Coarse	2888	191	61	27	279	97
Total	Coarse	86550	2739	870	822	4431	51
1503	Medium	4074	156	17	7	180	45
5489	Medium	2621	161	45	25	206	78
5492	Medium	11732	574	80	33	687	59
Total	Medium	18427	891	142	66	1073	58
5489	Fine	6700	255	75	33	363	54
5492	Fine	866	45	6	—	51	59
Total	Fine	7566	300	81	33	414	55

TABLE 10

Frequencies of changes from heavy variegation to light variegation.

PEDIGREE	PATTERN OF HEAVY VARIEGATION	NUMBER OF KERNELS OBSERVED	CHANGES TO LIGHT VARIEGATIONS EXPRESSED IN KERNELS AS INDICATED BELOW					
			¼	½	1	> 1	Total	Number per thousand
1501	Coarse	40975	190	108	66	2	366	9
1502	Coarse	7800	72	14	6	–	92	12
1503	Coarse	21497	92	25	25	–	142	7
Total	Coarse	71272	354	147	97	2	600	8
1503	Medium	4074	3	2	2	–	7	2